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The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., JUNE, 1932 NO. 9

SYMPOSIUM ON HEMATOLOGY

THE TECHNIC OF A BLOOD EXAMINATION*

RUSSELL L. HADEN, M.D., CLEVELAND, OHIO

IT MAY not appear to be necessary to preface a series of articles on hematology with one on the technic of a blood examination. Knowledge of the blood dyscrasias is dependent, however, on the data obtained from a study of the blood. The presence of an anemia, leucemia or other blood disease may be surmised, but no clinician would hazard an absolute diagnosis or outline a course of treatment without the aid of the laboratory.

It is important to have at hand as complete laboratory data as possible, and still more important to make sure that these findings are absolutely accurate. Too often the clinician is called upon to express an opinion based on blood films which are poorly made or unsatisfactorily stained, or on a blood picture for which the data is incomplete or evidently inaccurate. The selection of the best technical methods is difficult for those who are not constantly studying the problems of hematology.

The above considerations are my excuse for attempting in this article to outline a scheme of blood examination and to suggest satisfactory technical methods for the elucidation of the simpler problems of hematology.

A routine blood count (red and white cell count, hemoglobin estimation and differential count) is only a starting point for a more complete blood study and should be looked upon largely as a means of determining whether or not a complete blood study is indicated. In every case of anemia the following examinations should be done:

1. Red corpuscle count.
2. Determination of the mass of packed corpuscles.
3. Hemoglobin estimation.

*From Cleveland Clinic.

4. Calculation of indices:
   a. Volume index (erythrocyte volume relative to normal) or mean corpuscular volume.
   b. Color index (erythrocyte hemoglobin relative to normal) or mean corpuscular hemoglobin content.
   c. Saturation index (concentration of hemoglobin per unit volume of packed cells relative to normal) or mean corpuscular hemoglobin concentration.
5. White corpuscle count.
6. Study of stained blood film (size, shape, staining reactions and abnormalities of red cells, differential count of white cells, relative number of platelets).
7. Count of reticulocytes.
8. Determination of bile pigment content of the plasma.

These examinations are all necessary and are very easily done. I much prefer to make all examinations, except the study of the stained film, on blood withdrawn from a vein. The blood film alone is made from a drop of blood obtained from the ear lobe or the finger tip. A simple method for the entire ex-

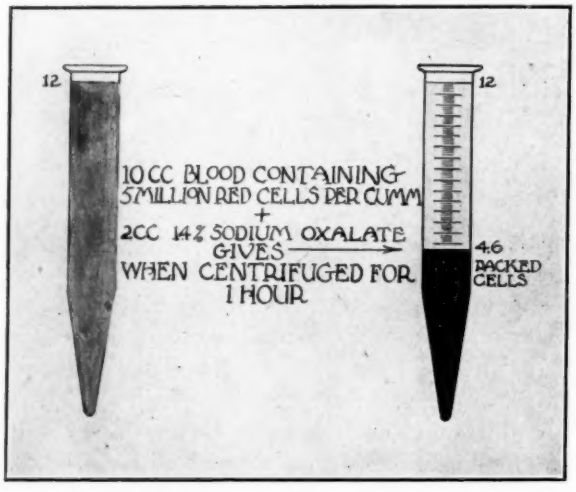


Fig. 1.—Centrifuge tube used for determination of mass of red blood cells.

amination is as follows: 20 c.c. of blood is withdrawn by means of a syringe, and exactly 10 c.c. is run into a 12 or 15 c.c. centrifuge tube, containing exactly 2 c.c. of 1.4 per cent sodium oxalate solution.[1] This is mixed by inverting and is then spun in a large centrifuge for one hour at 2500 revolutions per minute. The remainder of the blood is added to an ounce bottle containing one drop of a 30 per cent solution of potassium oxalate. The latter specimen is used for the red and white cell count and for the hemoglobin determinations. The examinations are made as indicated below.

1. Red Cell Count.—One source of inaccuracy in erythrocyte counts is the use of a hypotonic diluting fluid. I prefer to use a 0.9 per cent sodium chloride solution as the diluting agent. Accurate erythrocyte counts require much practice and experience on the part of the technician. It is absolutely necessary that accurately calibrated counting chambers and pipettes be used. These should be certified by the United States Bureau of Standards.

2. Volume of Packed Red Cells.—This is read off directly from the tube after centrifuging. The volume is recorded as the number of cubic centimeters of cells per 100 c.c. of blood, and in per cent of normal. The normal is calculated for each laboratory by determining by means of the centrifuge the number of cubic centimeters of packed cells per 100 c.c. of blood in normal individuals with a red cell count of 5 million cells per c.mm. With our present apparatus we have found 45 c.c. of cells per 100 c.c. of blood to be equal to 100 per cent (Fig. 1). For any given specimen of blood the number of cubic centimeters of packed cells obtained by centrifuging 10 c.c. of blood is read off on the

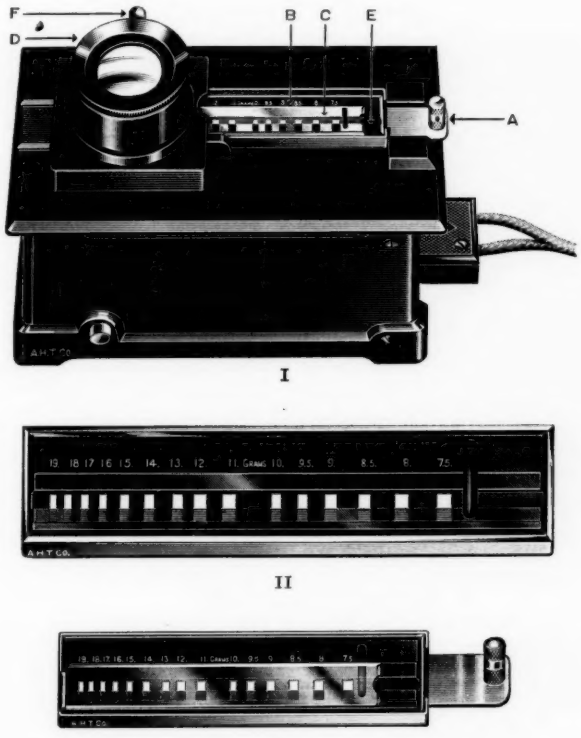


Fig. 2.—Haden-Hausser hemoglobinometer. I. Complete instrument. *A*, movable carrier; *B*, comparator slide; *C*, cover glass; *D*, reading microscope; *E*, wedge-shaped channel; *F*, light shutter. II. Comparator slide. III. Comparator slide with cover glass in metal holder.

tube and divided by 4.5 c.c. (or other figure determined as normal for the 10 c.c. of blood).

3. Hemoglobin Estimation.—Accurate hemoglobin determinations may be made quite easily by the oxygen capacity method, using the Van Slyke apparatus, or by one of the iron methods. Such procedures are not practical, however, in routine clinical work. The exact number of grams of hemoglobin present in any given blood is of no great clinical importance. It is exceedingly important, however, to determine the hemoglobin content relative to normal. This can be done simply if a hemoglobinometer reading directly in grams is used for

the determination. The new Sahli, the new Dare, the Bausch and Lamb-Newcomer, the Klett, the old Miescher-vonFleischl, and the Haden-Hausser[2] instruments, all read in grams although no two give the same reading on the same specimen of blood. This makes little difference if every one in his own laboratory determines for the instrument used the average number of grams of hemoglobin per 100 c.c. of blood in normal individuals with a red cell count of 5 million per c.mm., and takes this as 100 per cent. The results are then always reported, not in the absolute number of grams per 100 c.c., but in per cent of normal. In normal individuals the color index is always 1.00 within the limits of error. By this method the percentage of hemoglobin for a given specimen of blood would always be the same in all laboratories although the actual number of grams of hemoglobin determined would be different in each. In our laboratories the Haden-Hausser hemoglobinometer (Fig. 2) which reads only in grams is used routinely and 15.4 grams of hemoglobin is taken as 100 per cent.

4. Calculation of Indices.—By the methods outlined we are now able to determine accurately the red cell count and the packed cells in per cent of normal, packed cells (normal equals the number of cubic centimeters of packed cells found in 100 c.c. of normal blood with a red cell count of 5 million) and the hemoglobin in per cent of normal hemoglobin (normal equals the number of grams of hemoglobin found in 100 c.c. of normal blood with a red cell count of 5 million). Suppose for a given laboratory a specimen of normal blood with a red cell count of 5 million per c.mm. yields 46 c.c. of packed cells per 100 c.c. on centrifuging with an isotonic anticoagulant for one hour at 2500 revolutions per minute, and contains 15 grams of hemoglobin per 100 c.c., and a specimen of anemic blood with a red cell count of 1.5 million yields 18.4 c.c. of packed cells, and contains 6.0 grams of hemoglobin, then:

(a) The volume index (volume of average cell relative to normal)

$$\text{of the normal blood} = \frac{\dfrac{\text{Number of c.c. of packed cells found per 100 c.c.}}{\text{Normal number of c.c. of packed cells per 100 c.c.}}}{\dfrac{\text{Number of red cells found}}{\text{Normal number of red cells}}} = \frac{\dfrac{46}{46}}{\dfrac{5{,}000{,}000}{5{,}000{,}000}} = 1.00$$

$$\text{of the anemic blood} = \frac{\dfrac{18.4}{46}}{\dfrac{1{,}500{,}000}{5{,}000{,}000}} = 1.33$$

The mean corpuscular volume[3] (the volume of the average red corpuscle in cubic microns) is calculated by dividing the volume of packed cells per 100 c.c. by the number of cells contained in 100 c.c. of blood. The result may be calculated in cubic microns by multiplying by 2 the volume of packed cells per 100 c.c. per 5,000,000 cells.

Thus the mean corpuscular volume of the normal blood = $46 \times 2 = 92$ cubic microns; of the anemic blood = $\frac{\frac{18.4}{1{,}500{,}000}}{\frac{}{5{,}000{,}000}} \times 2 = 61.3 \times 2 = 123$ cubic microns.

(b) The color index (amount of hemoglobin per cell relative to normal)

$$\text{of the normal blood} = \frac{\frac{\text{Number of grams of hemoglobin found per 100 c.c.}}{\text{Normal number of grams of hemoglobin}}}{\frac{\text{Number of cells found per c.mm.}}{\text{Normal number of red cells per c.mm.}}} = \frac{\frac{15}{15}}{\frac{5{,}000{,}000}{5{,}000{,}000}} = 1.00$$

$$\text{of the anemic blood} = \frac{\frac{6.0}{15.0}}{\frac{1{,}500{,}000}{5{,}000{,}000}} = 1.33$$

The mean corpuscular hemoglobin[2] (the hemoglobin content of the average red corpuscle in micromicrograms) is calculated by dividing the hemoglobin in grams per 100 c.c. of blood by the number of cells contained in 100 c.c. of blood. It is simply calculated in micromicrograms by multiplying by 2 the number of grams of hemoglobin per 100 c.c. of blood per 5 million cells.

Thus the mean corpuscular hemoglobin of the normal blood = $15.0 \times 2 = 30$ micromicrograms of the anemic blood = $\frac{\frac{6.0}{1{,}500{,}000}}{\frac{}{5{,}000{,}000}} \times 2 = 20 \times 2 = 40$ micromicrograms.

(c) The saturation index (amount of hemoglobin per unit volume of cell relative to normal)

$$\text{of the normal blood} = \frac{\frac{\text{Number of grams of hemoglobin found in 100 c.c.}}{\text{Normal number of grams of hemoglobin per 100 c.c.}}}{\frac{\text{Number of c.c. of packed cells found per 100 c.c.}}{\text{Normal number of c.c. of packed cells per 100 c.c.}}} = \frac{\frac{15}{15}}{\frac{46}{46}} = 1.00$$

$$\text{of the anemic blood} = \frac{\frac{6}{15}}{\frac{18.4}{46.0}} = 1.00$$

The mean corpuscular hemoglobin concentration[3] (the concentration of the hemoglobin in per cent per unit volume of cells) is calculated by dividing the number of grams of hemoglobin per 100 c.c. of blood by the number of cubic centimeters of packed cells per 100 c.c.

Then the mean corpuscular hemoglobin concentration in the normal blood $= \frac{15}{46} = 32.6$ per cent, in the anemic blood $= \frac{6.0}{18.4} = 32.6$ per cent.

The calculation of the different indices is facilitated by the use of a nomogram (Fig. 3).

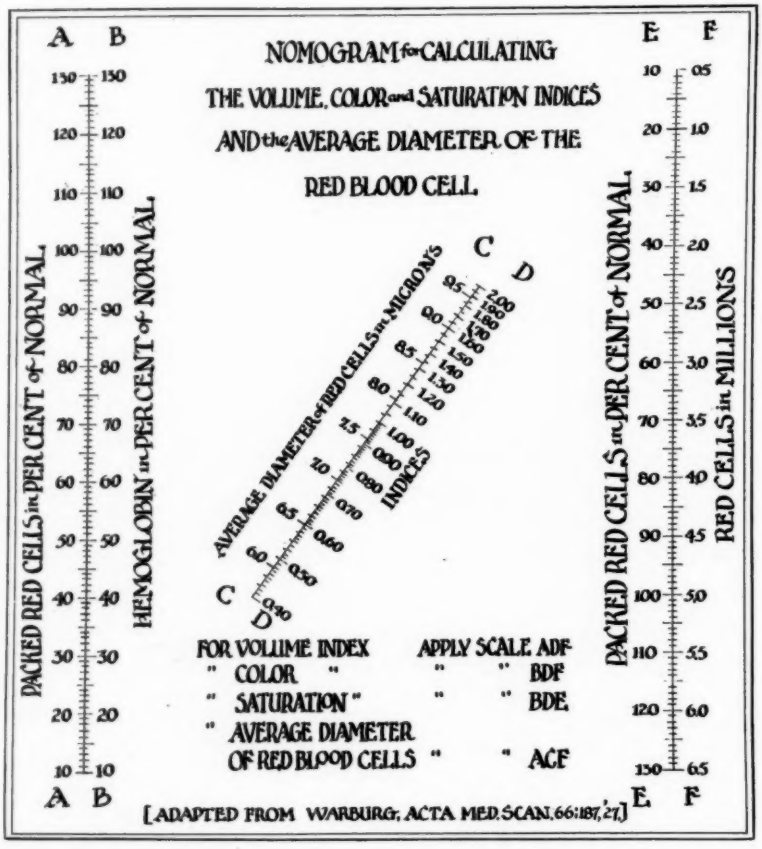

NOMOGRAM for CALCULATING
THE VOLUME, COLOR and SATURATION INDICES
AND the AVERAGE DIAMETER OF THE
RED BLOOD CELL.
PACKED RED CELLS in PER CENT of NORMAL
HEMOGLOBIN in PER CENT of NORMAL
AVERAGE DIAMETER of RED CELLS in MICRONS
INDICES
PACKED RED CELLS in PER CENT of NORMAL
RED CELLS in MILLIONS
FOR VOLUME INDEX APPLY SCALE ADF
" COLOR " " " BDF
" SATURATION " " " BDE
" AVERAGE DIAMETER OF RED BLOOD CELLS " " ACF
[ADAPTED FROM WARBURG, ACTA MED. SCAN. 66:187, '27.]

Fig. 3.—Nomogram for calculating indices from red cell count, hemoglobin in per cent of normal and packed red cells in per cent of normal. The mean diameter of the red blood cells can also be calculated.

5. White Corpuscle Count.—This is subject to fewer errors and greater variation than is the red cell count but should be equally carefully done.

6. Preparation of Stained Film.—In many laboratories blood films are made only on slides. For the study of the morphology of the red cells, for reticulocyte counts and for examinations for parasites, such films are satisfactory. For an accurate differential count, for determining the relative number of platelets, and for studying the morphology of the white cells, films made on cover glasses are far preferable. The technic of a blood examination is certainly not mastered until one can make satisfactory cover glass preparations. These are easily made

if certain precautions are observed. I find no difficulty in having the best of preparations made by efficient technicians.

The most satisfactory cover glasses are No. 2, $\frac{7}{8}$ inch square of good manufacture. They must be absolutely clean and free from dust. Some cleaning solution such as a concentrated acid or bichromate acid mixture is often employed. The best method of cleaning is to scrub them with some grit-free scouring powder such as Dutch Cleanser. The hands are thoroughly washed, a number of cover glasses placed in the palm of one and the scouring powder and a

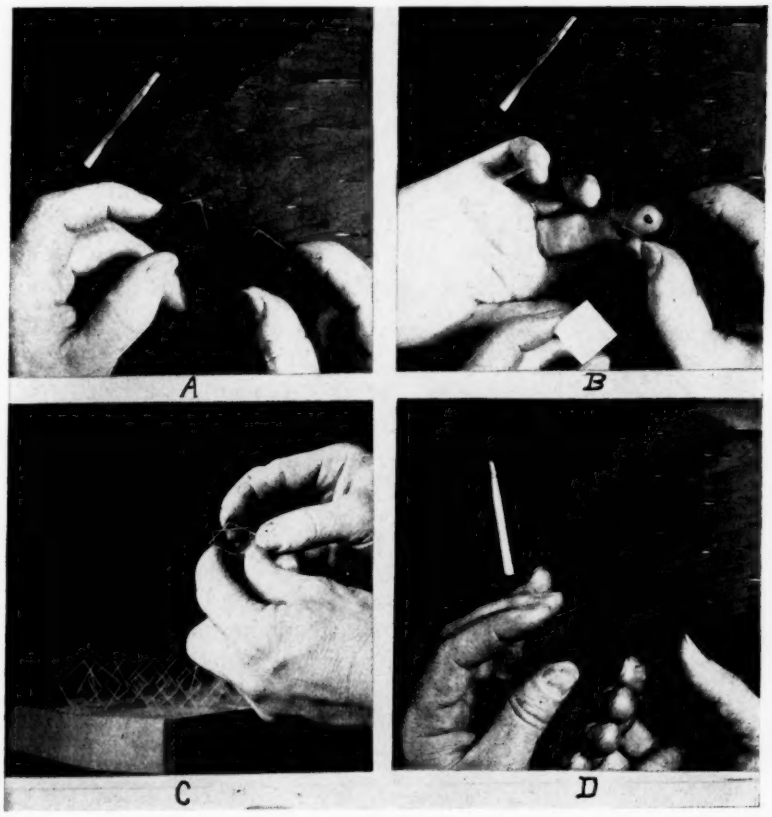


Fig. 4.—The preparation of blood films by the cover glass method. *A*, a cover glass (⅞ inch square No. 2) is grasped at the adjacent corners with the thumb and forefinger of each hand; *B*, the drop of blood is touched with the cover glass held in the right hand; *C*, the cover glass carrying the drop of blood is quickly placed parallel on the cover glass held in the left hand; *D*, cover glasses are then drawn apart with a sliding motion, care being taken to keep them parallel. The films are allowed to dry in air and are then ready for staining. The drop of blood must be globoid on the finger tip and just large enough to cover the cover glass when properly spread. (From Haden—*Clinical Laboratory Methods.*)

small amount of water added. The glasses are then well scrubbed with the palm of the other hand using a rotary motion. They are rinsed with distilled water, placed in alcohol, dried with a clean, lint-free cloth, and stored in boxes. Just before use they are brushed off with a camel's hair brush and placed on edge in a block of wood or in the top of a box. We usually make a number of slits in the top of a 20 c.c. syringe box and keep in the box an automatic lancet, the box of cleaned cover glasses, a camel's hair brush, cotton gauze and a small bottle of alcohol, thus providing everything needed for making blood films.

In making the films, a clean and dust-free cover glass is grasped at the adjacent corners with the thumb and forefinger of each hand and the drop of blood on the finger tip is touched with the cover glass held in the right hand (Fig. 4). The cover glass carrying the drop of blood is quickly pressed parallel to the cover glass held in the left hand. The blood spreads by capillary attraction. As the spread is completed the cover glasses are drawn apart with a sliding motion, care being taken to keep them parallel. The films are allowed to dry in air and are then ready for staining. The finger is punctured with an automatic lancet since the depth of the puncture wound can be regulated best in this manner. The drop of blood must be globoid on the finger tip and just large enough to cover the cover glass when properly spread.

Staining the Blood Film.—The films are best stained on a small stand made by nailing a row of corks to a wood block (Fig. 5). Wright's stain is the most satisfactory one for routine use. Only chemically pure, acetone-free methyl alcohol such as Merck's Blue Label should be employed in making the staining solution. Cover the blood film with about 10 drops of stain and after one minute add an equal number of drops of distilled water. Very often preparations made in this manner are too blue due to an excess of alkali in the stain. The

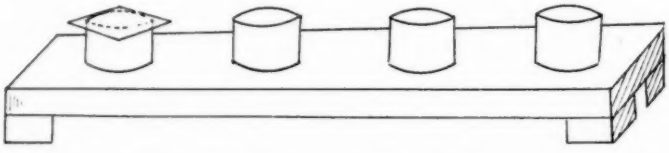


Fig. 5.—Convenient stand for staining blood films made on cover glasses. (From Haden—*Clinical Laboratory Methods.*)

simplest way to correct an excess of alkalinity is by adding a phosphate buffer solution. The optimum amount of buffer solution to be added must be determined by trial. Usually the most satisfactory stains are made by adding 3 drops of a phosphate buffer solution with a P_H equal to 6.4 and 8 to 10 drops of distilled water. If the staining solution is very alkaline, only the buffer solution is used. Let stand for four to five minutes. The phosphate buffer solution with P_H equal to 6.4 is made as follows.

Primary potassium phosphate (KH_2PO_4)	6.63 gm.
Anhydrous secondary sodium phosphate (Na_2HPO_4)	2.56 gm.
Distilled water to make	1000.00 c.c.

The stained films are mounted film side down in neutral gum damar solution. A rather thin solution of gum damar in chemically pure zylol is made, calcium carbonate is thoroughly mixed with it and the solution placed in the window in the sunlight for several weeks. After the calcium carbonate has completely settled out, the solution is poured off and placed in a warm place until it has evaporated to the proper consistency. The gum damar thus made is neutral, does not darken with age, and does not cause fading of the stain.

7. Count of Reticulocytes.—The reticulocytes may be stained with brilliant cresyl blue in a number of different ways. Often a film of cresyl blue is pre-

pared on cover glasses and the blood film made on this. We prefer the following technic. A drop of a saturated solution of brilliant cresyl blue in alcohol is placed on a porcelain drop plate (Fig. 6) and allowed to evaporate to dryness. One drop of the blood taken from the centrifuge tube prepared for the determination of the red cell volume is mixed with the stain. This is taken up with a pipette. Films are prepared on cover glasses and counterstained with Wright's stain.

If only a reticulocyte count is to be made, a drop of blood from the finger tip is taken up with a capillary, mixed with the dried stain in the drop plate, and blood films made from the mixture.

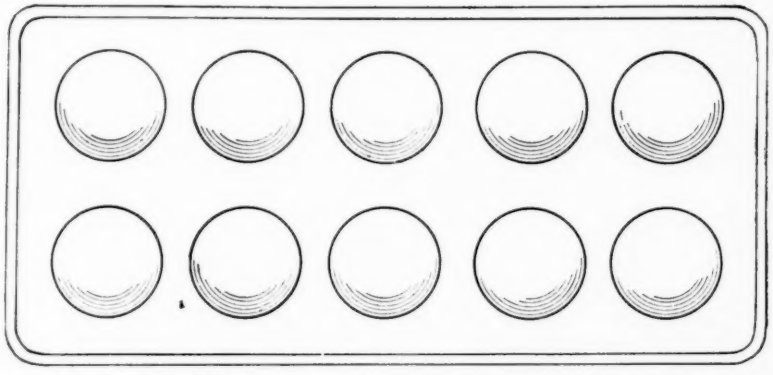


Fig. 6.—Porcelain mixing plate for use in blood grouping. (From Gradwohl and Blaivas—*Blood and Urine Chemistry.*)

8. Determinations of Bile Pigment Content of the Blood Plasma.—The bile pigments are easily and satisfactorily estimated as the icterus index. I use the method suggested by Murphy.[4] For the color comparison, a series of standards are prepared from various dilutions made from a 1:100 solution of potassium bichromate to correspond with varying icterus index figures as shown in Table I.

TABLE I

DILUTION	CORRESPONDING ICTERUS INDEX	DILUTION	CORRESPONDING ICTERUS INDEX
1:10,000	1	1:500	20
1:5,000	2	1:400	25
1:2,000	5	1:200	50
1:1,000	10	1:133	75
1:666	15	1:100	100

The solutions are kept in a rack in small test tubes 10 mm. in diameter (Fig. 7). One or two cubic centimeters of the supernatant plasma is pipetted from the centrifuge tube after spinning into a similar test tube and compared with the bichromate standards. The figure corresponding to the dilution which matches the serum is the icterus index of the serum. A correction is made for the dilution with oxalate. The normal icterus index is 4 to 6. To avoid clouding, blood should be taken when the patient is fasting. In preparing the dilutions of potassium bichromate, 2 drops of concentrated sulphuric acid should be added to each 500 c.c. to prevent fading.

To Recapitulate.—Twenty cubic centimeters of blood have been taken from the patient's vein and blood films have been made from the finger tip. Ten cubic centimeters of blood have been mixed with isotonic sodium oxalate. Before centrifuging, films for a count of the reticulocytes have been made from a drop of the oxalated blood. After centrifuging, the volume of red cells has been read off and the icterus index has been determined on the supernatant plasma. Red cell and white cell counts have been made on the specimen to which a drop

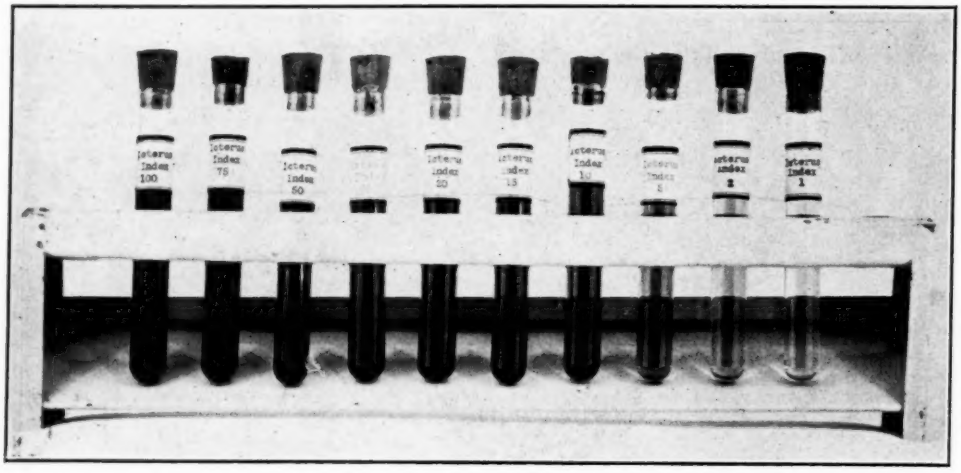


Fig. 7.—Set of bichromate standards for estimating the icterus index.

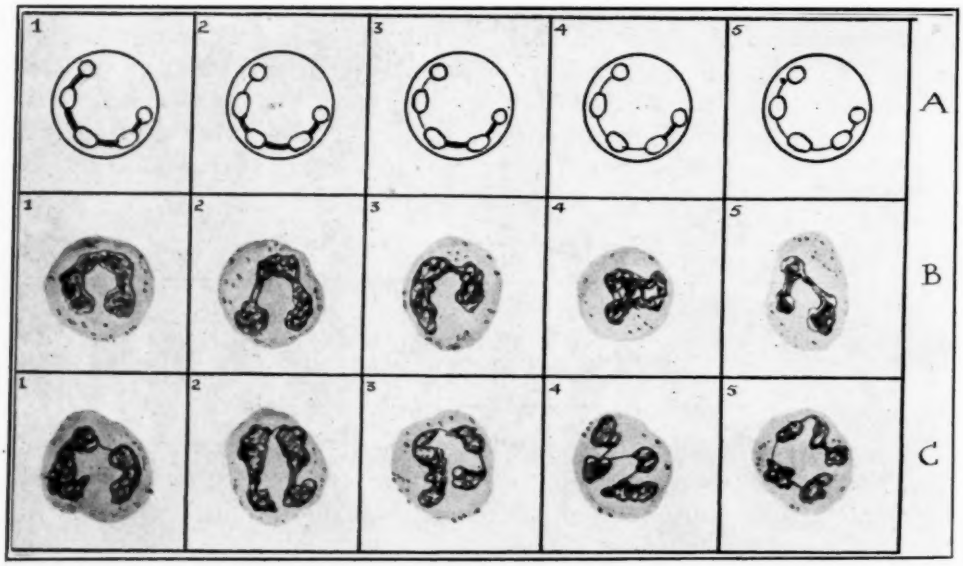


Fig. 8.—A diagram to illustrate different types of nuclei in polymorphonuclear neutrophilic cells. *1*, polymorphonuclear with nucleus of five lobes connected by thick bands of nuclear tissue. The nucleus shows five distinct masses but since the connecting threads are thick, the cell is designated nonfilamented. *2, 3, 4, 5*, polymorphonuclears in which two or more lobes are connected only by a filament. These four cells are all designated "filamented" polymorphonuclears. *B, 1, 2, 3, 4, 5*, nonfilamented polymorphonuclears. In each cell the lobes of the nucleus are connected by thick threads. *C, 1, 2, 3, 4, 5*, filamented polymorphonuclears. In each cell two or more lobes are connected only by a filament of nuclear tissue. (Adapted from Cooke and Ponder's—*The Polynuclear Count.*)

of potassium oxalate has been added. The blood film has been stained and examined and the indices have been calculated from the data obtained above. A complete examination has thus been made with a minimum expenditure of time and trouble. Tests other than those outlined above may be indicated. The more common one of definite value is a *special study of white cells.*

a. For Maturity: Numerous classifications to indicate the maturity of the polymorphonuclear cells, based on a study of the nucleus have been suggested (Arneth, Schilling, Cooke and Ponder, Pons and Krumbhaar). In my opinion the most satisfactory and practical classification is the separation of the polymorphonuclear neutrophiles into two groups, filamented and nonfilamented, as suggested by Farley, St. Clair and Reisinger.[5] Such counts can be made only on well prepared and properly stained blood films on cover glasses. One hundred polymorphonuclear neutrophiles are counted. Cells in which the lobes of the nucleus are connected only by a thin strand or filament of nuclear material are counted as filamented cells. If there is any band of nuclear material except this chromatin filament connecting different parts of the nucleus, such a cell is counted as nonfilamented (Fig. 8). If 100 polymorphonuclear cells are counted not more than 25 per cent should be nonfilamented. If only 100 white cells of all types are counted not over 16 per cent of the neutrophiles should be nonfilamented.

Any irregularity in size and staining reactions of the granules should be noted, since such changes are a good index of the degree of toxicity and may be equally important as variations in maturing of the nucleus.

b. For Oxidase Content: This is of value in differentiating cells of the lymphocyte and bone marrow series. I think the best method is a slight modification of the Goodpasture[6] stains. The following stock stain is kept on hand:

Alcohol, 95 per cent	100.00 c.c.
Sodium nitroprusside	0.05 gm.
Benzidine, c. p.	0.05 gm.
Basic fuchsin	0.05 gm.

The sodium nitroprusside is dissolved in 1 to 2 c.c. of water and added to the alcohol in which the benzidine and fuchsin have been dissolved. Ten drops of the reagent are poured on the blood film, allowed to remain two minutes, and then diluted with an equal quantity of phosphate buffer solution ($P_H = 6.4$) containing 0.5 per cent hydrogen peroxide added just before use.

c. Jenner-Giemsa Stain for Special Study of Leucocytes: The films stained by Wright's method are satisfactory for most purposes. The Jenner-Giemsa stain brings out beautifully the finer details of nuclear and other cell structures. In leucemia especially, such preparations are valuable. They are made as follows:

The cover glass preparation is covered with Jenner's stain for three minutes, and an equal number of drops of distilled water added. After one minute, the stain is washed off. The cover glass is then placed with the film down in a watch glass. The Giemsa stain (15 drops of the stock Giemsa solution to 10 c.c. of distilled water) is run into the watch glass from the side and left for from ten to fifteen minutes. Wash, dry, and mount in neutral gum damar.

SPECIAL EXAMINATIONS INDICATED IN HEMORRHAGIC DISEASES AND OTHER CONDITIONS

1. Platelet Count.—An excellent idea of the relative number of platelets may be gained from an examination of a properly made cover glass preparation. If the number seems diminished a count should be done. The Rees-Ecker method is a very satisfactory one. A small amount of diluting fluid (sodium citrate, 3.8 grams, formalin, 0.2 c.c., brilliant cresyl blue, 0.1 gram, distilled water 100 c.c.) is drawn into the bulb of the diluting pipette to moisten the capillary. The blood is then drawn up to the 0.5 mark and the bulb filled with the diluting fluid. The counting and calculation is done as for a red cell count.

2. Determination of Fragility of Erythrocytes.—The method described for this by Giffin and Sanford[7] is a simple and satisfactory one (Fig. 9). Twelve

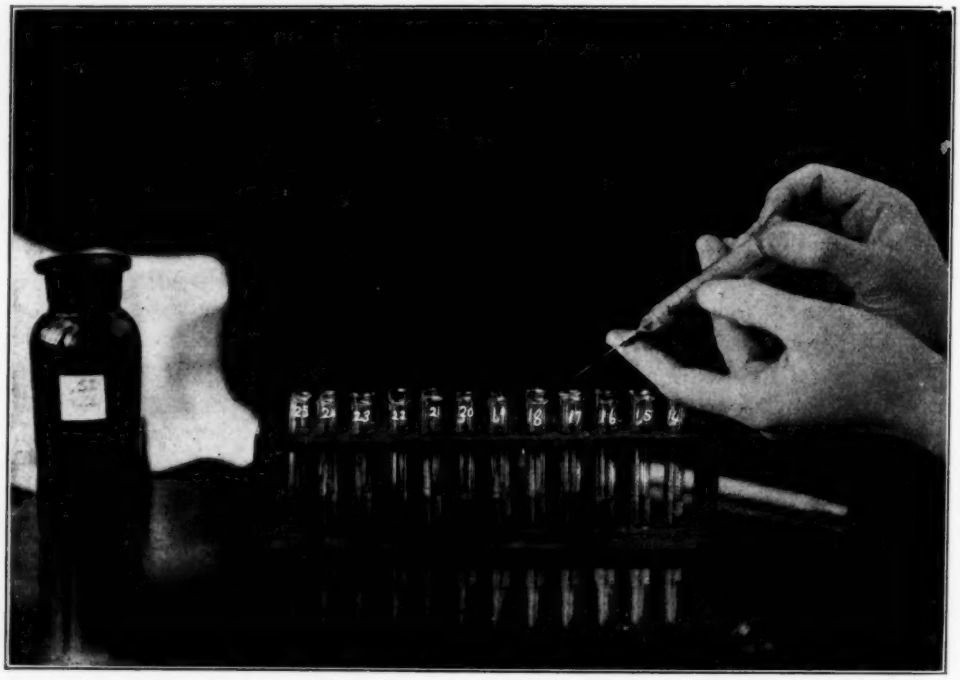


Fig. 9.—Method for determination of fragility of red blood cells. One drop of whole blood is added to each tube of hypisotonic solution. (After Giffin and Sanford.)

Wassermann tubes are set up in a rack and numbered 25 to 14 from left to right, with a capillary pipette run into each tube, the number of drops of an accurately made solution of 0.5 per cent sodium chloride being indicated by the figure on the tube. Distilled water is added by means of the same pipette to make the total number of drops of an accurately made solution of 0.5 per cent sodium chloride indicated by the figure on the tube. Distilled water is added with the same pipette to bring the total number of drops in each tube up to 25. Blood is withdrawn from a vein by means of a dry sterile syringe and one drop run into each tube. The tubes are allowed to stand at room temperature for one hour or more. The dilution in which there is just a slight tingeing of the supernatant fluid due to laking of a few of the least resistant corpuscles is noted as the point of initial hemolysis. Reading from left to right, complete hemolysis

is indicated in the first tube in which no corpuscular residue is evident by shaking the tube.

The percentage of sodium chloride in any tube is calculated by multiplying the number on the tube by 0.02. Normal blood shows intense hemolysis in 0.42 or 0.38 per cent sodium chloride solution, and complete hemolysis in 0.36 to 0.32 per cent.

3. Determination of Coagulation Time.—It is a waste of time to determine the coagulation time on a drop of blood obtained by piercing the skin. The method suggested by Lee and White[8] is a satisfactory one for clinical use. Blood is withdrawn from a vein with a syringe in which the space between the end of the plunger and the needle is filled with salt solution, and one cubic centimeter is run into each of 3 small Wassermann tubes 8 mm. in diameter. The tubes should be scrupulously clean and washed with salt solution just before use. After standing for three minutes a tube is rotated endwise every thirty seconds, and that point at which the blood no longer flows from its position but maintains its surface contour when inverted, is taken as the end point. Normal blood coagulates by this method in five to eight minutes.

4. Bleeding Time.—This is easily and quickly done by the method of Duke.[9]

The blood report form is a convenient one on which to report the results of the examination.

BLOOD REPORT

Name: Date:
Case No: Service:

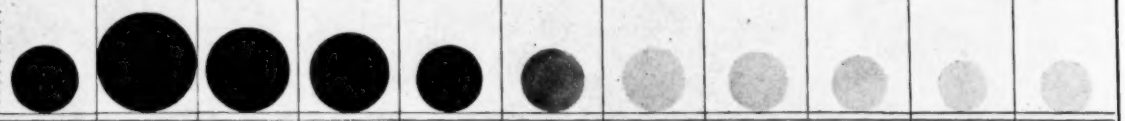
1. RED BLOOD CELLS:
   1. Number per c.mm.—
   2. Size in stained preparation—
   3. Shape in stained preparation—
   4. Color in stained preparation—
   5. Regeneration forms:
      (a) Nucleated red cells—
      (b) Basophilia: punctate or diffuse—
      (c) Nuclear particles—
   6. Fragility: hemolysis begins in % : complete in %
      sodium chloride (normal)
   7. Reticulocyte count—
2. VOLUME OF PACKED RED BLOOD CELLS % of normal (c.c. per 100 c.c.)
3. VOLUME INDEX (Mean corpuscular volume = cubic microns)
4. HEMOGLOBIN % of normal (gm. per 100 c.c. with hemoglobinometer)
5. COLOR INDEX (Mean corpuscular hemoglobin = micromicrograms)
6. SATURATION INDEX (Mean corpuscular hemoglobin concentration = per cent)
7. WHITE BLOOD CELLS:
   1. Number per c. mm.—
   2. Differential count:
      Neutrophiles— % Eosinophiles— % Basophiles— %
      Lymphocytes— % Monocytes— %
      Nonfilamented neutrophiles— % (normal 6-16%)
   3. Presence of abnormal forms:
      (a) Myelocytes— (c) Lymphoblasts
      (b) Myeloblasts— (d) Fragile leucocytes
      (e) Toxic neutrophiles
8. BILE PIGMENTS IN PLASMA:
   (a) Icterus index (Normal 4 to 6)
   (b) Units (van den Bergh) per 100 c.c. (Normal 0.5 to 2 units)
9. PLATELETS per c.mm.
10. COAGULATION TIME (method)
11. REMARKS
12. LABORATORY DIAGNOSIS:

Name of Examiner———————————

CLASSIFICATION of ANEMIA on NUMBER VOLUME AND HEMOGLOBIN CONTENT OF ERYTHROCYTES

→ NORMAL ← TYPES OF ANEMIA →

MEAN CORPUSCULAR VOLUME IN PER CENT OF NORMAL (RELATIVE CORPUSCULAR HEMOGLOBIN INDICATED BY INTENSITY OF SHADING)



INDICES	NUMBER INDEX	0.90-1.10	<0.80	<0.80	<0.80	<0.90	<0.90	0.90-1.10	>1.10	0.90-1.10	<0.90	>1.10
	VOLUME INDEX	0.90-1.10	>1.10	>1.10	>1.10	0.90-1.10	0.90-1.10	0.90-1.10	0.90-1.10	<0.90	<0.90	<0.90
	COLOR INDEX	0.90-1.10	>1.10	0.90-1.10	<0.90	0.90-1.10	<0.90	<0.90	<0.90	<0.90	<0.90	<0.90
	SATURATION INDEX	0.90-1.10	0.90-1.10	<0.90	<0.90	0.90-1.10	<0.90	<0.90	<0.90	VARIABLE	VARIABLE	VARIABLE
TYPICAL EXAMPLE	CELL COUNT	5 MILLIONS	2.0	3.0	2.50	3.50	3.20	5.00	5.60	5.00	3.50	5.60
	VOLUME INDEX	1.00	1.50	1.25	1.20	1.00	0.96	0.95	0.90	0.80	0.70	0.70
	COLOR INDEX	1.00	1.40	1.00	0.84	0.95	0.70	0.60	0.60	0.60	0.50	0.50
	SATURATION INDEX	1.00	0.93	0.80	0.70	0.95	0.73	0.63	0.55	0.75	0.71	0.71
CLASSIFICATION OF BLOOD		NORMOCYTHEMIC NORMOCYTIC AND NORMOCHROMIC BLOOD	HYPOCYTHEMIC MACROCYTIC AND HYPERCHROMIC ANEMIA	HYPOCYTHEMIC MACROCYTIC AND NORMOCHROMIC ANEMIA	HYPOCYTHEMIC MACROCYTIC AND HYPOCHROMIC ANEMIA	HYPOCYTHEMIC NORMOCYTIC AND NORMOCHROMIC ANEMIA	HYPOCYTHEMIC NORMOCYTIC AND HYPOCHROMIC ANEMIA	NORMOCYTHEMIC NORMOCYTIC AND HYPOCHROMIC ANEMIA	HYPERCYTHEMIC NORMOCYTIC AND HYPOCHROMIC ANEMIA	NORMOCYTHEMIC MICROCYTIC AND HYPOCHROMIC ANEMIA	HYPOCYTHEMIC MICROCYTIC AND HYPOCHROMIC ANEMIA	HYPERCYTHEMIC MICROCYTIC AND HYPOCHROMIC ANEMIA

Chart 1.—Showing classification of anemias on number, volume and hemoglobin content of red blood cells.

THE LABORATORY CLASSIFICATION OF ANEMIA ON THE BASIS OF VOLUME AND HEMOGLOBIN CONTENT

The laboratory classification of anemia has always been unsatisfactory. A rough differentiation into primary and secondary types is very frequently used. The anemias having a color index greater than 1.00 are usually classified as primary and those having a color index of 1.00 or less as secondary. Hampson and Shackle[10] first suggested the classification of anemias on the basis of cell size, using the terms "megalocytic" and "nonmegalocytic." Wintrobe[11] suggested four groups: (1) macrocytic, (2) normocytic, (3) simple microcytic, and (4) hypochromic. Certainly the most logical laboratory classification is based on all three variants of the erythrocyte, namely, number, size, and hemoglobin content. The following terms may well be employed to indicate variations which have been observed in these factors.

Number	Hypercythemic	= red cell count > normal
	Normocythemic	= red cell count within normal limits
	Hypocythemic	= red cell count < normal
Volume	Macrocytic	= mean corpuscular volume > normal (VI > 1.10)
	Normocytic	= mean corpuscular volume = normal (VI = 0.90-1.10)
	Microcytic	= mean corpuscular volume < normal (VI < 0.90)
Hemoglobin Content	Hyperchromic	= Mean corpuscular hemoglobin > normal (C I > 1.10)
	Normochromic	= Mean corpuscular hemoglobin = normal (C I = 0.90-1.10)
	Hypochromic	= Mean corpuscular hemoglobin < normal (C I < 0.90)

All the different types of anemia which may occur from this standpoint are:

Normocythemic	Normocytic and hypochromic
	Microcytic and hypochromic
Hypercythemic	Normocytic and hypochromic
	Microcytic and hypochromic
Hypocythemic	Macrocytic and hyperchromic
	Macrocytic and normochromic
	Macrocytic and hypochromic
	Normocytic and normochromic
	Normocytic and hypochromic
	Microcytic and hypochromic

These different types of anemia are illustrated in Chart 1. The circles indicate relative volume, not diameter, and the intensity of color indicates the relative hemoglobin content. A typical example of each type of anemia is given. Every anemia should be thought of in terms of number, volume and hemoglobin content of the average erythrocyte, and every case should be classified on such criteria. An anemia with a red cell count of 3.50 millions, a volume index of 0.75 and a color index of 0.65 is reported as a hypocythemic, microcytic and hypochromic anemia rather than simply as "secondary" anemia. Likewise an anemia with a count of 2 millions and a volume and color index of 1.50 is recorded as hypocythemic, macrocytic and hyperchromic rather than "primary."

SUMMARY

I have tried to emphasize the need for an accurate and complete examination of the blood in studying hemotologic problems. Any clinician who has had the opportunity of utilizing such an examination will never be satisfied with any other kind. A complete blood study may be made quickly and simply in a well equipped laboratory.

I have indicated one satisfactory technic for each of the tests suggested, although others may be equally satisfactory. One good method should be used until it is thoroughly mastered.

Only with such laboratory data can an accurate knowledge of the blood dyscrasias be gained.

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THE VALUE OF ACCURATELY DETERMINED COLOR, VOLUME AND SATURATION INDEXES IN ANEMIAS*†

Based on a Study of Over Two Hundred Patients

Edwin E. Osgood, M.D., Howard D. Haskins, M.D., and Frank E. Trotman, M.D., Portland, Oregon

RECENT developments[1] in the therapy of anemias have greatly increased the importance of accurate differential diagnosis. The value of the color index in differentiating between pernicious and other anemias was learned long ago; but the use of inaccurate methods and incorrect standards for calculation have prevented the full realization of its possibilities. The more recently introduced volume and saturation indexes have been far less thoroughly studied, but appear to have equal or greater diagnostic value.

The perfecting of a simple hemoglobin method[2, 3] of research accuracy, a uniform system of hematologic methods[4] for use with oxalated venous blood, and the establishment of normal standards[5, 6, 7, 8] for comparison has made possible the present restudy of the value of these indexes in the differential diagnosis of anemias. This study has revealed far more diagnostic value for these indexes than we had dared to hope.

REVIEW OF THE LITERATURE

Apparently Johannes Duncan[9] (1867) was the first to recognize the possibility of variation in size and in hemoglobin content of the red cells in different diseases, thus forming a clear concept of the information which the color, volume and saturation indexes give. although he did not actually determine them. He did demonstrate the relative decrease in hemoglobin content and size of the red cells in chlorosis.

The color index was first calculated by Hayem[10, 11] (1878). Leeuwenhoek[12] (1673) was apparently the first to measure the size of red cells by their diameter, but Welcker[13] (1864) was the first to make clinical application of the method. He demonstrated that the blood corpuscles in chlorosis were smaller than normal.

Sørensen[14] (1876) was apparently the first to point out the fact that the increase in size of the red cells is one of the most characteristic features of pernicious anemia, but the popular spread of this knowledge was due largely to Paul Ehrlich[16] (1880) and to Lauche[17] (1883). Bleibtreu and Bleibtreu[18] (1892) were the first to work out a clinically practical method for determining the red cell volume, but did not apply it to hematology. Credit for the introduction of the hematocrit for the determination of red cell volume belongs to Hedin[19] (1890) and Blix, Daland[20] (1891), and Gaertner[21] (1892). Herz[23] (1893) was the first to use a quantitative expression for the ratio of hemoglobin to cell volume which he called the "specifische Hämoglobingehalt." He divided the hemoglobin estimation by the total cell volume, thus securing a figure corresponding to the hemoglobin index of Whipple and Robscheit-Robbins.[30] He had a full understanding of the various

*From the Departments of Medicine and Biochemistry of the University of Oregon Medical School.

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possible relationships between cell count, volume, and hemoglobin content. He reports results on several cases.

By the end of the nineteenth century the color index was in common use and findings in different types of anemia had been fairly thoroughly studied. Methods were available for the determination of cell volume and diameter and a few students of the anemias knew of the more important changes in cell size and hemoglobin content which occur. The volume index and saturation index had not been calculated and very few individuals had any clear concept of the changes in the red cells which these indexes express. Full reviews of this early literature will be found in the monographs of Eichhorst,[15] Laache,[17] Reinert,[22] and in the writings of Ehrlich, Lazarus, and Naegeli.[24]

In 1901, Capps[25] first reported the method for calculation of the volume index and in 1903,[26] he reported detailed studies on the blood of 175 persons, 10 of whom were normal. These studies included hemoglobin estimations by the Fleischl method, red cell counts, cell volume determinations by the hematocrit* method, computation of the average diameter of the red cells based on the measurement of 100 cells with the eyepiece micrometer, and the calculation of the color and the volume index. Had his methods been as accurate as those of today, undoubtedly he would have discovered almost everything that is now known of the value of these indexes. The failure to recognize the importance of this work has postponed for at least twenty-five years the attainment of the accuracy in diagnosis of anemias which a full understanding of his researches makes possible. Notwithstanding the fact that with the technic used he could not have obtained full packing of the red cells, that his hemoglobin estimations cannot be transformed into absolute values, and that he based his normal standards for comparison on the study of only four men and six women, he arrived at the following conclusions, all of which have since been confirmed:

"1. The centrifuge accurately determines the mass of red corpuscles, but cannot be relied upon to estimate the number of cells, because the volume of the cell undergoes variations in disease.

"2. The volume of the individual erythrocyte is best obtained by using the centrifuge in conjunction with the hemacytometer. Volume Index is an expression used to designate the volume of the erythrocyte relative to the normal. Measurement of diameters for the determination of cell size is of limited value and often misleading, especially when poikilocytosis is present.

"3. The cell volume is invariably increased in pernicious anemia and usually more so than the Hb content of the cell. This heightened volume index is a more constant and trustworthy sign of pernicious anemia than the increased color index. The polychromemia in pernicious anemia is due to an increase in cell volume and not to an increased affinity of the protoplasm for Hb.

"5. In a large proportion of chlorotics the cell volume suffers as well as the Hb, although always to a less extent. The volume index is of great significance in prognosis. . . .

"7. The Hb content of a normal erythrocyte, as indicated by a color and volume index of 1.00, represents the point of saturation of the protoplasm. When, therefore, the color index rises above 1.00, we assume that a corresponding increase has taken place in the cell volume (except in jaundice, where the color test is unreliable). Supersaturation of cell protoplasm with Hb probably does not occur. On the other hand, the cell may lose Hb without necessarily losing in volume.

"8. Cell volume seems to be chiefly altered by influences affecting cell growth or degeneration. The large erythrocytes of pernicious anemia are probably young cells. Small cells may result from a malnutrition of the bone marrow, as in chlorosis, or from an actual degeneration, as in sepsis.

"9. The cell volume suffers remarkably little change from osmotic influences according to my observations. Dropsy, cyanosis, the hydremia following acute hemorrhage, and jaundice (with some exceptions) do not materially alter the volume of the cells."

His data permit many conclusions which he did not report.

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*He centrifugated at 10,000 revolutions per minute for three minutes, relying only on speed of manipulation to secure packing of the cells before clotting occurred.

Wroth[27] (1907) studied eleven cases of anemia, but added nothing to the work of Capps. In 1911, Larrabee[28] studied 139 individuals, 21 of whom were normal, but his methods were so inaccurate that his results and conclusions are incorrect in many cases. Alder[29] (1918), using a viscosimetric method for the determination of cell volume, studied eleven cases of anemia. He was apparently the first after Herz[23] to use a quantitative method for expressing the relationship of the hemoglobin to the cell volume, the ratio now expressed by the saturation index. He simply divided the hemoglobin percentage by the cell volume per 100 c.c., considering 2.2 to 2.4 as the limits of normal variation. This ratio corresponds to the hemoglobin index of Whipple and Robscheit-Robbins.[30] Alder was the first to state that red cells tend to approach the spherical form in hemolytic icterus. He confirmed Capps's observation that the concentration of hemoglobin in the red cell is not increased in pernicious anemia, but that the increased color index is due to the increased average cell volume and that the hemoglobin content of the red cell is low in chlorosis.

Bönninger[31] (1919) was the first to calculate the saturation index in essentially its present form. He called it the Färbeindex-Volumen in contradistinction to the color index which he called Färbeindex-Zahl. He gave cell volume figures in terms of cubic micra of the average red cell instead of in terms of the volume index, but the latter may readily be calculated from his data. In a previous paper, he studied results on sixty normal persons and he adds twenty in this paper. He was thus the first to study any adequate series of normals. His red cell counts and hemoglobin estimations are obviously inaccurate, but his cell volume determinations were much better than any previously made. His average figures for total volume of packed red cells per 100 c.c. of blood, namely, 44.7 c.c. for men and 41.0 c.c. for women, would stand today. He reports results on 61 patients with various diseases, being the first following Capps to give data of clinical value on an extensive series of patients. He confirmed most of the statements of Capps and Alder.

Gram[32] (1920) reported very briefly his conclusions from volume index studies on 611 persons.

Suzuki[33] (1920) and Reich[34] (1921) review and compare methods for the determination of cell volume. Reich was also apparently the first to use oxalated blood for hematologic study. He reports results, including color and saturation indexes (Färbeindex-Volumen), on 33 patients with various diseases, 15 of whom he inadvisedly used as normals. His methods were so inaccurate, however, that his figures have very little value.

Csaki[35] (1922; claims work was completed in 1919) was the first to emphasize centrifugation to a constant volume rather than for a definite time interval. He calculated color, volume and saturation indexes (Färbeindex-Volumen). Had he had an adequate series of strict normals for comparison, his results would have been more accurate than those of any of his predecessors. Unfortunately, he based his normals on the study of 23 hospital patients. He reports results on 47 patients with various diseases and confirms most of the points mentioned by Capps[26] and Bönninger.[31] Many of his conclusions do not seem warranted by the data presented and have not been borne out by subsequent investigations.

Carrie Frochlich[36] (1922) reviewed the literature and studied 85 healthy persons and 64 patients. She published results of color indexes on 65 normals and 28 patients and results of color and saturation indexes (Färbeindex-Volumen) together with the average volume of one red cell on an additional 20 normals and 36 patients. Her technic, calculations, and method of selecting normals were far more satisfactory than those of any of her predecessors. Her figures are the first to show that the red cell is slightly larger in women (by 1.3 per cent) than in men.

Rossdale[37] (1923) reports results of studies on a few patients using unsatisfactory methods. His conclusions are difficult to correlate with his data.

In 1923, while the present study was in progress, Haden's[38] work appeared. This study was based on 40 normal men, 12 normal women, 20 cases of pernicious anemia, and 32 patients which he classed as having secondary anemia. His determinations were all done on oxalated venous blood and the hemoglobin estimations were far more accurate than those of his predecessors. However, he neglected to centrifugate to constant volume, and it has since been shown that the time and speed of centrifugation which he used are not sufficient

for complete packing of the red cells in all cases. Apparently unaware of the previous work on the saturation index (Färbeindex-Volumen), he coins this term and gives directions for calculating this index, which Capps[26] had so nearly discovered (see paragraph 7 of his conclusions quoted above). Most of the current interest in the color, volume and saturation indexes dates from this article by Haden.

Drucker[39] (1924) studied the hemoglobin, cell volume, and saturation of the cell with hemoglobin in 270 healthy children and 217 children with various diseases. Unfortunately, he did not do red cell counts.

In 1924, Haden[40] republished his previous results with the addition of 30 cases of pernicious anemia and 20 of secondary anemia.

In 1926, Osgood[5] suggested a tentative classification of anemias by color, volume and saturation indexes, based on the study of the first 63 cases included in the present paper. In this classification, the diagnostic value of a low saturation index in anemias of chronic blood loss was first pointed out.

Jørgensen and Warburg[41] (1926) give a very valuable review of the literature on the size and hemoglobin content of the red cell. They report color, volume and saturation indexes with measurement of cell diameter on 7 normal persons and 45 patients. The most complete bibliography of this subject yet published is appended.

TABLE I.

	RED CELLS MILLION PER C.MM.			HEMOGLOBIN GRAMS PER 100 C.C.			CELL-VOLUME C.C. PACKED CELLS PER 100 C.C. OF BLOOD		
	AVERAGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVERAGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVERAGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES
Men (196)	5.4	4.46 to 6.40	4.70 to 6.10	15.80	13.44 to 20.11	14.00 to 18.00	44.79	36.09 to 51.87	40.10 to 48.97
Women (106)	4.8	4.14 to 5.55	4.30 to 5.30	13.70	10.98 to 16.49	12.00 to 15.50	41.00	35.32 to 45.89	37.20 to 45.00

Greppi[42] (1927) reports color and volume indexes, together with total blood and plasma volume determinations, on 6 patients with pernicious anemia and 12 patients with secondary anemia.

Cameron[43] (1928) reports color and volume indexes and grams of hemoglobin per 100 c.c. of packed cells on 10 normal persons, 10 cases of pernicious anemia, and 25 other patients, drawing the conclusion that "in pernicious anemia the hemoglobin content of a given volume of red blood corpuscles is invariably above the average normal value, and usually much above this value." This statement is directly contrary to the statement of Capps, Haden, and others that supersaturation of the cell with hemoglobin does not occur.

Some time after the first presentation[44] (July, 1929) of the data and conclusions included in this paper, Wintrobe[45, 46] (May, 1930) published preliminary reports of studies on 140 patients with anemia. He[45] suggests a classification very similar to that previously suggested by one of us[5] (1926).

SELECTION OF SUBJECTS

More than 200 patients* were selected for study because they had been diagnosed as anemic by some one. Time did not permit the study of all such patients available during the seven years over which this research extended,

*These were from the Multnomah County Hospital and from the private practices of Drs. Harold C. Bean, I. C. Brill, N. W. Jones, Laurence Selling, and others.

so our figures have no value for a determination of the relative frequencies of different types of anemia.

All cases were discarded from this series in which an almost certainly complete and correct diagnosis was not *ultimately* made by criteria other than the color, volume and saturation indexes and agreed to by other physicians seeing the case. This left a group of 167 examinations on 144 cases which will be discussed in detail.

METHODS*

The clinical examinations were carefully checked by one of the authors. Oxalated venous blood was used for the hematologic methods.[4] Gastric contents analyses, stool examinations, and uranalyses including tests for urobilinogen, were done in almost all cases.

Red cell counts were made with research accuracy, using Toisson's diluting fluid and Bureau of Standards apparatus. The counts reported are the

NORMALS

COEFFICENTS		INDEXES								
HEMOGLOBIN	VOLUME	COLOR			VOLUME			SATURATION		
AVERAGE	AVERAGE	AVERAGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVERAGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVERAGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES
14.66	40.70	1.00	0.84 to 1.21	0.90 to 1.12	1.00	0.78 to 1.12	0.90 to 1.08	1.00	0.90 to 1.23	0.90 to 1.10
14.34	42.83	1.01	0.83 to 1.19	0.90 to 1.10	1.00	0.86 to 1.11	0.90 to 1.10	1.00	0.87 to 1.16	0.90 to 1.10

average of two or more dilutions agreeing within 100,000. Hemoglobin estimations were made by the Osgood-Haskins method.[2, 3] Cell volume determinations** were made by the method[5] previously described by us. Centrifugation was carried out until the cell volume remained constant, a precaution which has been neglected by many other workers. A correction of 3.5 per cent of the volume of the packed red cells must be added if it is desired to compare the results with those in which an isotonic anticoagulant was used. Control study showed each of these three methods to have limits of error of plus or minus 2 per cent.

Calculation of Indexes.—The normal standards (Table I) used were determined by the authors on 196† healthy young men[5] and 106† healthy young

*All details of the methods used may be found by reference to the *Textbook of Laboratory Diagnosis* by Edwin E. Osgood and Howard D. Haskins. P. Blakiston's Son & Co., Inc., Philadelphia.

**We consider that Ponder's[48] study of our method of determining cell volume is a confirmation of its accuracy as his average on ten cases differs from our average on 153 cases by less than twice the probable error of his results.

Haden[49] in his recent excellent review of red cell volume determinations in man now recognizes the importance of centrifugation to constant volume, but criticizes the use of a hypertonic anticoagulant. The justice of this criticism is recognized, but we think that the advantages[4] far outweigh the disadvantages.

†Since the original papers were published, hematologic studies of fifty-nine healthy men and six healthy women have been added to the series without significantly changing any of the figures reported.

women,[6] using the same technic and apparatus as that used in the present research. Our standards have been shown[7, 8] to agree closely with the averages of all reliable studies made to date.

The indexes were calculated as follows:

$$\text{Color Index} = \frac{\text{Per cent Hemoglobin,}}{\text{Per cent Red Cells}}$$ when the *hemoglobin coefficient** for normal persons (for men, 14.7; for women, 14.3) of the patient's sex and age group is taken as 100 per cent hemoglobin and when 5 million per c.mm. is taken as 100 per cent red cells. It expresses the ratio of the hemoglobin per unit number of cells in the patient's blood to the average hemoglobin per unit number of cells in the blood of normal persons of the patient's sex and age group.

$$\text{Volume Index} = \frac{\text{Per cent Cell Volume,}}{\text{Per cent Red Cells}}$$ when the *volume coefficient*** for normal persons (for men, 41; for women, 43) of the patient's sex and age group is taken as 100 per cent cell volume and when 5 million per c.mm. is taken as 100 per cent red cells. It expresses the ratio of the mean size of the cells in the blood examined to the mean size of the cells in the average blood of normal individuals of the patient's sex and age group.

$$\text{Saturation Index} = \frac{\text{Per cent Hemoglobin}}{\text{Per cent Volume}},$$ when the hemoglobin coefficient and the volume coefficient for normal persons of the patient's sex and age group are taken as 100 per cent hemoglobin and cell volume, respectively. It expresses the ratio between the hemoglobin per unit volume of cells in the blood examined and the average hemoglobin per unit volume of cells in the blood of healthy persons of the same sex and age group.

In the present study all calculations were made with five place logarithms, but for clinical use a table and a chart[47] have been prepared which greatly simplify these calculations.

A smear stained with Wright's stain was studied and saved for reference in each case. Other hematologic studies such as white and differential cell counts, icterus index determinations, platelet and reticulocyte counts, fragility tests, peroxidase stains, etc., were made as indicated. Whenever possible, an autopsy was performed on those patients who died.

RESULTS

Untreated Pernicious Anemia.—In Table II are shown the results of 43 blood examinations on 37 cases (20 men and 17 women) of untreated pernicious anemia. Patients 3 and 10 were unusually young for pernicious anemia, but in Case 3 the diagnosis was confirmed by necropsy and in Case 10 the achlorhydria, combined system disease, leucopenia, and normal red cell fragility seemed to us sufficient evidence to exclude the former diagnosis of familial hemolytic icterus and to establish the diagnosis of pernicious anemia. This patient died, but no necropsy was secured.

A therapeutic test was not applied in most cases because they were studied

*The *hemoglobin coefficient* is a term introduced by the authors for "the grams of hemoglobin per 100 c.c. of blood calculated to a red cell count of 5 million per c.mm."

**The *volume coefficient* is a term introduced by the authors for the "volume of packed red cells per 100 c.c. of blood calculated to a red cell count of 5 million per c.mm."

before the discovery of "liver" (nuclear) therapy. Free hydrochloric acid was not found in the stomach contents of any case reported as pernicious anemia.

Note, in Table II, the uniformly high color and volume indexes with normal saturation indexes. This completely confirms the statement of Capps,[26] Bönninger,[31] Haden,[38] and others that the fundamental alteration in the red cell in pernicious anemia is an increase in size with a corresponding increase in hemoglobin content. In no case is there a true hyperchromia as is claimed by Cameron[43] and as is stated in most of the older texts.

TABLE II

UNTREATED PERNICIOUS ANEMIA

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C. MM.	HEMOGLOBIN PER CENT**	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX
1	M	52	0.51	16.6	2.29	7.00	1.54	1.69	0.91
2	M	38	0.42	16.7	2.31	7.14	1.89	2.09	0.90
*3	M	24	0.53	16.8	2.32	6.58	1.50	1.53	0.98
4	F	60	0.64	18.5	2.55	7.18	1.39	1.31	1.07
5	M	57	0.53	18.5	2.55	7.79	1.64	1.79	0.91
6	F	65	0.45	18.9	2.61	9.35	2.04	2.43	0.84
			1.21	39.9	5.51	18.75	1.59	1.80	0.88
*7	F	49	0.64	20.9	2.88	7.56	1.59	1.38	1.15
8	M	66	0.95	28.6	3.94	10.91	1.41	1.40	1.01
9	F	68	1.01	29.5	4.07	11.34	1.41	1.31	1.08
10	F	16	1.02	34.7	4.79	14.33	1.64	1.63	1.01
11	F	77	0.80	30.1	4.15	10.27	1.82	1.50	1.22
12	M	62	1.02	37.2	5.14	13.00	1.71	1.55	1.10
13	F	71	1.14	38.8	5.35	13.11	1.64	1.33	1.23
14	F	59	1.18	40.2	5.55	14.84	1.64	1.46	1.12
15	M	44	1.33	40.7	5.62	15.36	1.44	1.41	1.02
16	F	72	1.39	41.9	5.79	15.89	1.45	1.33	1.09
17	M	75	1.28	42.8	5.90	18.39	1.57	1.75	0.90
18	M	74	1.26	43.5	6.00	18.67	1.62	1.81	0.90
19	F	71	1.63	43.9	6.06	18.27	1.30	1.30	1.00
20	M	65	1.32	44.4	6.13	16.00	1.58	1.48	1.07
21	M	78	1.51	46.4	6.41	17.25	1.44	1.39	1.04
22	M	62	1.64	48.0	6.62	17.48	1.43	1.30	1.10
*23	F	50	1.77	50.7	7.00	19.58	1.38	1.29	1.07
24	F	57	1.62	52.0	7.18	18.89	1.55	1.36	1.14
25	M	57	1.99	55.5	7.66	20.81	1.31	1.28	1.03
			1.32	38.9	5.36	14.95	1.38	1.38	1.00
26	M	55	1.92	60.3	8.31	19.28	1.47	1.22	1.20
27	F	66	2.13	61.4	8.47	26.89	1.39	1.47	0.95
			2.09	64.1	8.85	25.71	1.48	1.43	1.03
28	M	61	2.66	63.3	8.73	27.52	1.12	1.26	0.88
29	M	56	2.40	64.9	8.95	26.84	1.24	1.36	0.91
30	F	67	2.28	66.5	9.18	27.69	1.41	1.41	1.00
31	F	66	2.76	72.0	9.94	32.50	1.26	1.37	0.92
			1.28	36.3	5.01	13.23	1.37	1.20	1.14
32	M	45	2.53	74.0	10.21	32.98	1.37	1.59	0.86
33	F	51	2.43	79.5	10.97	32.10	1.58	1.54	1.03
			2.58	80.7	11.14	32.79	1.51	1.48	1.02
34	M	67	2.91	82.0	11.32	33.16	1.32	1.39	0.95
35	F	70	3.21	84.2	11.62	32.23	1.27	1.17	1.08
36	M	66	3.06	88.0	12.14	36.21	1.35	1.44	0.94
37	M	49	3.71	88.7	12.24	39.73	1.12	1.31	0.86
			2.87	81.8	11.29	32.83	1.35	1.40	0.96
Minimum		16	0.42	16.6	2.29	6.58	1.12	1.17	0.84
Maximum		78	3.71	88.7	12.24	39.73	2.04	2.43	1.23
Average		59	1.65	48.9	6.75	19.59	1.48	1.47	0.99

*Diagnosis confirmed by necropsy.
**100 per cent hemoglobin is equivalent to 13.8 grams per 100 c.c. of blood.

Observe that 100 per cent of the diagnoses would have been correctly made if both the color and volume indexes were determined and if a result of over 1.25 in either were considered diagnostic of pernicious anemia. Only one case (35) would have been missed if the volume index alone had been used, and only two (28 and 37) if an accurate color index alone had been relied on. These facts should be kept in mind for comparison with the clinical diagnostic average on first examination which will be discussed later. The average color index is 1.48 with a range of 1.12 to 2.04. The average volume index is 1.47 with a range of 1.17 to 2.43. The average saturation index is 0.99, or almost exactly normal, and the range is 0.84 to 1.23 which is almost identical with the normal range as shown in Table I.

Study of the stained smears in some of these cases showed very few deviations from the normal, although it is possible that the tedious procedure of measuring the diameters of 1000 cells might have led to the diagnosis. Megaloblasts and nucleated red cells were found so inconstantly that it was evident that failure to find them cannot be used as evidence against the diagnosis of pernicious anemia. On the other hand, typical megaloblasts were found in cases of leucemia, lead poisoning, and carcinoma with metastases to the bone marrow, hence they are far from conclusive proof of the presence of pernicious anemia even when found.

It is noteworthy that in the cases (34 to 37) in which the hemoglobin is comparatively high, the color and volume indexes are still sufficiently high to be of diagnostic value.

TABLE III

TREATED PERNICIOUS ANEMIA

CASE NO.	SEX	AGE	DAYS OF TREATMENT	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C. C.	VOLUME OF CELLS PER 100 C. C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX
*11	F	77		*0.80*	*30.1*	*4.15*	*10.27*	*1.82*	*1.50*	*1.22*
			67	3.61	77.5	10.70	34.13	1.04	1.10	0.94
*33	F	51		*2.43*	*79.5*	*10.97*	*32.10*	*1.58*	*1.54*	*1.03*
			?	4.02	95.1	13.12	41.04	1.14	1.19	0.96
*34	M	67		*2.91*	*82.0*	*11.32*	*33.16*	*1.32*	*1.39*	*0.95*
			10	3.32	90.0	12.42	31.52	1.27	1.16	1.10
*38	F	37		*0.84*	*25.0*	*3.45*	*8.81*	*1.44*	*1.22*	*1.17*
			44	2.58	39.0	5.38	17.50	0.73	0.79	0.92
**12	M	62		*1.02*	*37.2*	*5.14*	*13.00*	*1.71*	*1.55*	*1.10*
			31	3.34	81.0	11.18	33.07	1.14	1.21	0.94
			64	4.54	85.2	11.76	38.61	0.88	1.04	0.85
**15	M	44		*1.33*	*40.7*	*5.62*	*15.36*	*1.44*	*1.41*	*1.02*
			24	2.43	81.5	11.25	30.77	1.57	1.54	1.02
			57	4.39	98.7	13.62	43.08	1.06	1.20	0.88
**37	M	49		---	---	---	---	---	---	---
			54	5.01	111.5	15.39	48.74	1.04	1.19	0.88
**39	M	67		---	---	---	---	---	---	---
			370	2.27	56.0	7.73	21.33	1.16	1.15	1.01

*Nuclear extract.
**Minot-Murphy diet.

Treated Pernicious Anemia.—Table III presents results of studies on 8 cases of pernicious anemia before and after receiving specific treatment. The figures in italics are the results before treatment. The first four cases (11, 33, 34, and 38) were treated with the nuclear extract of Jones, Phillips, Larsell, and Nokes.[50, 51] The last four (12, 15, 37, and 39) were treated with the liver diet of Minot and Murphy.[1] Note that the color and volume indexes return to normal after a few weeks on either treatment. No changes as marked as these were observed in spontaneous remissions of untreated cases. Knowing this, it is necessary to find out whether the patient has been taking such therapy before relying on a normal or low color and volume index to exclude the diagnosis of pernicious anemia.

Anemia of Chronic Blood Loss.—Table IV presents results of the study of ten cases in which chronic hemorrhage was the only discoverable factor tending to produce anemia. Note that there is a marked tendency for all of the indexes to be low. As will be shown later, this is the only type of anemia, with the exception of the chlorotic type, in which the saturation index is low. The highest saturation index is 0.91 (Case 45) and this may well have been due to the large blood transfusion nine days before.

In Table V are presented fifteen studies on thirteen cases in which chronic hemorrhage was the chief, although probably not the only, factor tending to produce anemia. The results are similar to those in Table IV. In only two cases is the saturation index well within normal limits and in one of these (Case 60) the duration of the bleeding was unknown, while in the other (Case 61) the duration was only two months and a second determination a week or so later, just after a hysterectomy had been performed, showed a decreasing index. *This low saturation index occurs so rarely in any other type of anemia that, in our opinion, chronic hemorrhage should be considered as the most probable cause of any anemia showing a saturation index below 0.85, until it has been definitely excluded.* This is a very important diagnostic point, for it is thus possible to reach a correct conclusion the first day the patient is seen, even though the bleeding may have stopped previously or though several days' observation may be necessary to detect its source. A high saturation index, on the other hand, is a point against an anemia being due largely to chronic loss of blood even though bleeding is occurring. The diagnostic value of this index was apparently overlooked prior to the publication of our preliminary report,[5] although it is clearly illustrated in Capps's article[26] (see his Table XIV) and also in the results of other writers on this subject. More recently, Wintrobe[46] has confirmed this observation. A low icterus index was characteristic of this group of cases.

Anemias Due to Infection.—It has been customary to group these under the heading of hemolytic anemias; but it appears to the authors that this is unjustifiable, since depression of bone marrow activity by circulating toxin and sensitization of red cells to normal blood destroying mechanisms probably play a part in the production of these anemias, also. The relative proportion of the anemia due to these different factors undoubtedly varies with different sites and types of infection and with varying virulence of the organism.

TABLE IV

CHRONIC HEMORRHAGE ONLY

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
*40	M	63	1.45	18.1	2.49	8.61	0.58	0.72	0.81	Papilloadenoma of bladder.
41	M	59	2.02	25.5	3.52	11.14	0.59	0.67	0.88	Hemorrhoids, 15 yr. duration.
42	F	57	2.45	27.0	3.73	14.93	0.53	0.71	0.75	Hemorrhoids, 30 yr. duration.
43	M	44	4.32	50.0	6.90	25.25	0.54	0.71	0.76	Hemorrhoids.
44	M	60	2.31	32.3	4.47	15.27	0.66	0.81	0.81	Extreme hematuria.§
45	F	32	2.64	47.3	6.53	21.30	0.86	0.95	0.91	Metrorrhagia secondary to uterine fibroids.**
46	F	30	4.21	58.5	8.07	28.47	0.67	0.79	0.85	Essential menorrhagia, 1 yr. duration.
47	F	24	4.73	58.5	8.07	28.92	0.60	0.71	0.84	Essential menorrhagia.
48	F	15	3.78	60.4	8.34	29.41	0.77	0.90	0.85	Essential metrorrhagia, 1 yr. duration.***
49	M	40	3.53	57.8	7.98	30.66	0.77	1.06	0.73	Gastric ulcer, history of tarry stools for many months.
Minimum			1.45	18.1	2.49	8.61	0.53	0.67	0.73	
Maximum			4.73	60.4	8.34	30.66	0.86	1.06	0.91	
Average			3.14	43.5	6.01	21.42	0.66	0.80	0.82	

§Source undetermined. Recovery followed spontaneous cessation.
*Diagnosis confirmed by necropsy.
**Large transfusion nine days before.
***Bleeding ceased eleven days before count.

TABLE V

CHRONIC HEMORRHAGE THE CHIEF FACTOR

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
*50	M	69	1.47	23.1	3.18	10.85	0.74	0.90	0.82	Carcinoma of stomach.
*51	M	59	3.20	28.5	3.93	16.46	0.42	0.63	0.67	Carcinoma of stomach.
*52	M	60	1.96	30.1	4.15	13.78	0.72	0.86	0.84	Carcinoma of stomach.
*53	M	72	2.56	30.9	4.27	16.63	0.57	0.79	0.72	Carcinoma of stomach.
54	M	43	2.70	33.9	4.68	16.84	0.59	0.76	0.78	Carcinoma of stomach.
*55	M	58	3.26	37.2	5.14	16.67	0.54	0.62	0.86	Carcinoma of stomach.
			3.78	41.7	5.76	21.78	0.52	0.70	0.74	
*56	M	52	2.64	39.8	5.49	20.45	0.71	0.94	0.75	Carcinoma of stomach.
57	M	54	3.08	36.0	4.97	18.73	0.55	0.74	0.74	Carcinoma of pancreas; melena.
58	M	51	2.28	40.3	5.56	19.61	0.83	1.05	0.79	Peptic ulcer; chronic infectious arthritis.
59	F	55	3.50	45.5	6.28	24.21	0.63	0.80	0.78	Bright red and dark blood in stools for six years; cause undetermined. Arthritis, too.
60	F	67	3.34	49.3	6.80	20.62	0.71	0.72	0.99	Carcinoma of rectum; duration of bleeding unknown.
61	F	50	3.48	62.5	8.73	24.62	0.87	0.82	1.05	Metrorrhagia secondary to fibroid uterus.**
			3.38	55.8	7.70	26.27	0.77	0.92	0.86	Abscessed teeth.
62	M	45	3.66	71.2	9.83	33.08	0.91	1.10	0.83	Chronic ulcerative colitis; stools streaked with blood.
Minimum			1.47	23.1	3.18	10.85	0.42	0.62	0.67	
Maximum			3.78	71.2	9.83	33.08	0.91	1.10	1.05	
Average			2.95	41.7	5.76	20.04	0.67	0.82	0.81	
Average of Tables IV and V			3.03	42.4	5.86	20.57	0.67	0.82	0.82	

*Diagnosis confirmed by necropsy.
**Hysterectomy between counts.

TABLE VI

INFECTION ONLY

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
63	M	16	0.81	20.5	2.83	7.18	1.19	1.08	1.10	Chronic diffuse nephritis.
64	M	57	2.30	55.3	7.63	20.50	1.13	1.09	1.04	Chronic diffuse nephritis.
65	F	16	3.76	61.5	8.49	24.69	0.79	0.76	1.03	Chronic diffuse nephritis.
66	F	25	2.62	63.7	8.79	24.77	1.17	1.10	1.07	Chronic diffuse nephritis.
67	F	7	3.89	78.9	10.88	31.77	0.98	0.95	1.03	Early chronic diffuse nephritis.
68	M	29	2.84	52.3	7.22	22.75	0.86	0.98	0.88	Subacute glomerular nephritis; subacute septic arthritis of knee.
*69	M	48	3.23	67.0	9.25	23.46	0.97	0.89	1.10	Subacute glomerular nephritis, in uremia.
*70	M	54	1.74	44.0	6.07	17.59	1.18	1.23	0.96	Chronic cholecystitis and chronic fibrous peritonitis.
71	F	39	4.24	48.5	6.69	21.54	0.55	0.59	0.93	Chronic cholecystitis with stones. Ellipt. cells.
			4.10	44.8	6.18	20.59	0.53	0.58	0.90	
72	M	24	3.48	52.8	7.29	22.79	0.71	0.80	0.89	Subacute bacterial endocarditis with focal embolic nephritis.
			3.86	54.1	7.46	23.53	0.66	0.74	0.88	
*73	F	26	2.60	59.0	8.14	20.21	1.07	0.90	1.18	Subacute bacterial endocarditis; chronic diffuse nephritis.
74	M	52	2.69	60.2	8.31	27.34	1.05	1.24	0.85	Subacute bacterial endocarditis; hemolytic strep. found on blood culture.

*Diagnosis confirmed by necropsy.

TABLE VI—CONT'D

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
75	M	31	4.56	86.0	11.87	36.76	0.89	0.98	0.90	Subacute bacterial endocarditis; Strep. viridans found in blood.
*76	F	50	3.52	65.5	9.04	30.09	0.90	0.99	0.90	Endocarditis and bacteremia due to Staph. hemolyticus.
*77	M	76	2.49	55.0	7.59	23.25	1.04	1.14	0.91	Pyonephrosis with uremia secondary to prostatic obstruction.
78	F	37	4.71	61.5	8.49	29.15	0.63	0.72	0.88	Chronic sinusitis; history suggestive of cholecystitis; Graham-Cole neg. Ellipt. cells.
			4.72	62.3	8.60	31.12	0.64	0.77	0.83	
79	F	28	4.50	67.1	9.26	29.64	0.72	0.77	0.94	Chronic sinusitis; ellipt. cells.
80	M	77	3.08	67.4	9.30	30.18	1.03	1.19	0.86	Chronic sinusitis with "sinus" bronchitis.
81	F	26	4.34	67.4	9.30	31.05	0.75	0.83	0.90	Bilat. acute exudative tuberculosis; no cavitation; ellipt. cells.
82	F	18	3.48	76.6	10.57	30.02	1.06	1.00	1.06	Acute rheumtic fever with active mitral endocarditis.
83	M	58	4.17	87.2	12.03	36.62	0.98	1.07	0.92	Lobar pneumonia; nine days after crisis.
84	M	33	3.90	88.1	12.15	26.75	1.06	1.18	1.13	Luetic (arsenical?) hepatitis with jaundice.
*85	M	41	4.76	89.0	12.28	42.17	0.88	1.08	0.81	Subacute hepatitis; catarrhal jaundice.
Minimum			0.81	20.50	2.83	7.18	0.53	0.58	0.81	
Maximum			4.76	89.00	12.28	42.17	1.19	1.24	1.18	
Average			3.48	62.90	8.68	26.37	0.90	0.95	0.96	

In Table VI are presented twenty-six studies of twenty-three cases in which the anemia was due, as far as could be determined, solely to infection. They are arranged by disease groups. The first seven are cases of subacute glomerular or chronic diffuse nephritis. Our studies confirm the statements of Brown and Roth,[52, 53] namely, that anemia is almost constantly associated with this type of nephritis, that its severity runs parallel with the severity of the nephritis, and that the nature of the anemia (normal indexes, low reticulocyte count and icterus index, absence of urobilinogen in the urine) suggests as the chief etiologic factor bone marrow depression rather than hemolysis. Our results disagree with those reported by Ashe,[54] probably because he reports results obtained with the Dare hemoglobinometer which has been repeatedly shown to have limits of error exceeding plus or minus 35 per cent and thus is worse than valueless for color and saturation index calculations.

The normal saturation indexes confirm the experimental observations that the loss of blood cells in the urine is far too slight (always less than 5 c.c., usually less than 1 c.c., of blood per day) to be a factor in the production of the anemia. This anemia in chronic diffuse nephritis is so constant that a hematologic study is of great value in the differential diagnosis from hypertensive cardiovascular renal disease in which anemia rarely occurs.

The other conditions listed in this table require no special comment except to point out that these diagnoses should always be considered when searching for the cause of an anemia associated with normal or low color and volume indexes and with a normal saturation index.

Note that while the color and volume indexes may be either normal or low in this group, they never exceed the upper normal limits and that the saturation index is consistently normal, only two estimations below 0.85 being recorded.

Table VII shows results on eight cases in which infection was the chief factor in the production of the anemia. The results are similar to those in the previous table, but the saturation indexes average lower, due to the fact that hemorrhage was a complication in four cases.

Note the frequency with which elliptical erythrocytes were observed in these cases. Case 79 is the same as Adriana N. reported by Hunter and Adams.[55] They demonstrated similar elliptical cells in members of three generations of this same family, some of whom had no anemia. Lawrence[56] had previously reported the finding of elliptical and sickle-shaped erythrocytes in the blood of white persons. A search through our slides revealed typical elongated cells in certain cases of almost every type of anemia, hence we were forced to agree with Huck[57] that this is either merely one type of poikilocytosis or, which is more probable, that it is a familial anomaly of red cell form which is exaggerated by coexistent anemia but is not a factor in the production of that anemia. It is readily differentiated from true sickle-cell anemia which occurs only, as far as is known, in the negro race.

Anemia Associated With Diseases of the Blood-Forming Organs.—Table VIII presents results of fourteen studies on twelve cases. Note that, with few exceptions, the indexes are normal. In the one case (98) in which the color index was high, the white and differential cell count at once gave the

TABLE VII

INFECTION THE CHIEF FACTOR

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
86	F	22	2.63	34.5	4.76	15.75	0.63	0.70	0.91	Frontal sinusitis. Occasional epistaxis. Ellipt. cells.
*87	F	46	3.05	49.3	6.80	21.15	0.78	0.81	0.97	Sinusitis, cholecystitis, peptic ulcer with early carcinoma. Ellipt. cells.
88	F	21	3.97	47.3	6.53	22.66	0.57	0.66	0.87	Puerperal sepsis plus postpartum hemorrhage. Ellipt. cells.
89	F	46	4.22	54.0	7.45	25.74	0.62	0.71	0.87	Pharyngitis and bronchopneumonia. Pregnant at term. Ellipt. cells.
90	F	28	3.54	49.5	6.83	24.17	0.67	0.79	0.85	Chronic infectious arthritis. Amebic dysentery. (Diagnosed one year later.)
91	M	61	3.66	67.7	9.35	30.77	0.87	1.02	0.85	S. viridans bacteremia. Unexplained melena.
*92	M	38	3.50	75.4	10.40	33.33	1.01	1.16	0.87	S. viridans bacteremia, abscess of spleen, and subacute bacterial endocarditis. Bleeding hemorrhoids.
93	F	45	4.02	68.5	9.45	31.76	0.82	0.92	0.89	Tertiary syphilis. Ovarian cyst.
Minimum			2.63	34.5	4.76	15.75	0.57	0.66	0.85	
Maximum			4.22	75.4	10.40	33.33	1.01	1.16	0.97	
Average			3.57	55.8	7.69	25.67	0.75	0.85	0.88	
Average of Tables VI and VII			3.50	61.2	8.69	26.20	0.86	0.92	0.94	

*Diagnosis confirmed by necropsy.

TABLE VIII

DISEASES OF THE BLOOD-FORMING ORGANS

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
94	M	67	0.93	24.0	3.31	9.34	1.21	1.22	0.99	Chronic lymphoid leucemia.
95	F	49	1.90	40.8	5.63	18.16	1.01	1.17	0.86	Chronic lymphoid leucemia.
96	F	68	4.02	80.0	11.04	37.25	0.96	1.08	0.89	Chronic lymphoid leucemia.
97	M	57	3.58	92.3	12.74	33.95	1.21	1.16	1.05	Chronic lymphoid leucemia.
98	M	6	1.12	32.0	4.42	11.33	1.34	1.23	1.09	Acute myeloid leucemia.
*99	M	60	2.41	37.0	5.11	18.18	0.72	0.92	0.78	Acute myeloid leucemia. Syphilis.
100	M	26	2.52	48.8	6.73	20.83	0.91	1.01	0.90	Chronic myeloid leucemia.
*101	F	44	4.54	91.2	12.59	34.29	0.97	0.88	1.10	Chronic myeloid leucemia.
			3.50	85.5	11.80	35.44	1.18	1.18	1.00	
**102	F	13	2.51	57.0	7.87	24.07	1.10	1.12	0.98	Banti's disease.
			3.04	60.4	8.34	26.46	0.96	1.01	0.95	
**103	F	34	3.90	72.7	10.04	29.87	0.90	0.89	1.01	Banti's disease.
*104	F	36	4.38	71.5	9.87	32.35	0.79	0.86	0.92	Hodgkin's disease.
*105	M	57	3.27	81.3	11.22	28.95	1.17	1.08	1.08	Hodgkin's disease.
Minimum			0.93	24.0	3.31	9.34	0.72	0.86	0.78	
Maximum			4.38	92.3	12.74	37.25	1.34	1.23	1.10	
Average			2.97	62.5	8.62	25.77	1.03	1.06	0.97	

*Diagnosis confirmed by necropsy.
**Confirmed by pathological examination of spleen removed by operation.

differential diagnosis from pernicious anemia. There is some doubt whether these indexes are correct as adult standards were used for calculation since no satisfactory standards exist for children of this age (six years). In the one case (99) in which the saturation index was low, hemorrhage could not be excluded.

Miscellaneous Anemias.—Table IX contains twenty-eight studies on a very interesting group of nineteen cases of anemia of several types, the number of each type being too small to warrant a separate table.

The first two cases are the only chlorosis cases that we were able to find in a period of seven years. These were mild cases and the findings resemble those in the chronic hemorrhage group. In more severe cases Capps,[26] Bönninger[31] and others have found still lower color, volume and saturation indexes.

Malignant tumors (Cases 108 to 113) showed practically normal saturation indexes in contrast with the low indexes of carcinoma cases with hemorrhage (Table V). Case 111 deserves special mention as it was the only case with high indexes (aside from one leucemia) in which the diagnosis of pernicious anemia seemed doubtful. This patient was first seen by Dr. N. W. Jones and the diagnosis of multiple myeloma (proved correct at necropsy) was made by him, chiefly on the basis of the typical roentgen-ray findings in the bones. His laboratory also found a high color and volume index. There was nothing in the clinical picture either particularly to suggest or to rule out pernicious anemia. Necropsy revealed in addition to the multiple myeloma, a marked megaloblastic hyperplasia of the bone marrow, but the spleen was not enlarged and showed none of the changes common in pernicious anemia. We are undecided as to whether this is a coincidence of multiple myeloma and pernicious anemia, which seems very improbable, or whether the blood picture of pernicious anemia was produced by the involvement of the bone marrow. The latter would seem more probable were it not for the fact that the other malignant tumors in which the bone marrow was involved showed no elevation of the color and volume indexes. We have been unable to find reports of other cases of multiple myeloma in which sufficiently accurate hematologic studies were made to form a basis for conclusions. That anemia is associated as a rule with multiple myeloma has been shown[58] repeatedly.

The mode of production of anemia associated with malignant tumors is unquestionably varied and complex. Hemorrhage, bone marrow metastases, toxin production (?), and secondary infection may all play a part and the effects of toxin production and secondary infection may be local blood destruction, generalized hemolysis, or depression of bone marrow function. In our experience, hemorrhage and bone marrow metastases are by far the most important, since anemia occurs late or not at all in those tumors in which these can be definitely excluded.

The two cases (114 and 115) of malaria show normal indexes. This agrees with the observation of Wintrobe.[46] Here the chief factor in the production of the anemia is unquestionably the destruction of red blood cells within the blood stream. Similar findings were observed in one case (116) of lead poisoning and one case (117) of acetanilid poisoning. The case of lead poisoning is noteworthy in that as many as eighteen nucleated red cells,

TABLE IX

MISCELLANEOUS ANEMIAS

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
106	F	18	4.63	65.2	9.00	31.41	0.68	0.79	0.86	Chlorosis.
107	F	21	4.96	69.3	9.56	33.33	0.67	0.78	0.86	Chlorosis.
*108	M	65	2.33	38.1	5.25	17.78	0.77	0.93	0.82	Primary carcinoma of prostate with bone metastases, paraplegia, and sanguinopurulent cystopyonephrosis.
*109	M	64	2.58	43.8	6.04	18.85	0.80	0.89	0.89	Annular carcinoma of the rectum with bilateral hydronephrosis. No blood in stools.
*110	M	66	2.49	59.7	8.24	24.57	1.13	1.21	0.94	Malignant tumor of lymph glands metastasizing to bone.
*111	M	71	2.72	72.4	10.00	32.34	1.25	1.45	0.86	Multiple myeloma. (Pernicious anemia?)
112	F	71	4.75	78.0	10.76	32.26	0.79	0.79	1.00	Carcinomatosis. Primary site undetermined. No blood loss.
113	M	63	4.32	82.0	11.32	34.43	0.89	0.97	0.92	Scirrhous carcinoma of the stomach. No loss of blood.
114	M	32	3.46	67.9	9.37	29.68	0.92	1.05	0.88	Inoculation malaria for general paresis. Twenty days after inoculation.
115	M	18	3.38	73.5	10.14	30.41	1.02	1.10	0.93	Acquired tertian malaria.
116	M	32	2.96	59.0	8.14	22.61	0.94	0.93	1.00	Acute exacerbation of a chronic lead poisoning.

*Diagnosis confirmed by necropsy.

TABLE IX—CONT'D

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
			2.61	55.5	7.66	24.48	1.00	1.14	0.87	
117	M	47	3.67	79.2	10.93	34.74	1.01	1.15	0.88	Acetanilid poisoning.
*118	F	35	3.19	70.3	9.70	30.65	1.06	1.12	0.95	Polyglandular deficiency.
			3.14	67.9	9.37	30.77	1.04	1.14	0.92	
			3.75	84.7	11.69	37.04	1.09	1.15	0.95	
			0.92	22.7	3.14	8.98	1.19	1.14	1.05	
119	F	33	1.60	47.0	6.49	19.20	1.42	1.40	1.02	Pernicious anemia of pregnancy.
120	F	7	2.09	51.5	7.11	17.02	1.19	0.95	1.26	Sickle-cell anemia.
*121	M	67	1.73	41.6	5.74	16.78	1.13	1.18	0.95	Acute hemorrhage from duodenal ulcer. Carcinoma of head of pancreas.
122	M	47	2.75	59.2	8.17	22.82	1.01	1.01	1.00	Acute large hemorrhage from the bowel. Etiology undetermined.
123	F	19	2.83	66.3	9.14	25.00	1.13	1.03	1.10	Postpartum hemorrhage and acute bleeding hemorrhoids of twenty days' duration.
124	M	25	5.25	112.1	15.47	43.10	1.00	1.00	1.00	Normal six hours before donating 875 c.c. of blood.
			4.56	100.6	13.88	38.51	1.04	1.03	1.01	Twelve hours after donating blood.
			4.70	95.7	13.21	38.15	0.96	0.99	0.97	Eighty-four hours after donating blood.
			4.78	99.1	13.67	39.24	0.97	1.00	0.97	Eleven days after donating blood.
			4.99	102.0	14.08	39.49	0.96	0.97	0.99	Eighteen days after donating blood.
			4.92	111.8	15.43	42.89	1.07	1.06	1.00	Twenty-nine days after donating blood.

many of them typical megaloblasts, were seen in counting 100 white cells. This case would unquestionably have been diagnosed as pernicious anemia from a study of the stained smear had the criteria given in most texts been used.

Case 118* had a very interesting polyglandular deficiency in which hypofunction of the thyroid and suprarenal glands predominated. The counts extend over a period from February, 1925, to November, 1926. Note the very severe terminal anemia for which even at postmortem examination no cause other than the glandular deficiency could be found. Notwithstanding the statements, frequently made, that the anemia of myxedema may simulate that of pernicious anemia, her color and volume indexes remained normal throughout. This agrees with the observations of MacKenzie.[60]

Case 119 is our only case of pernicious anemia of pregnancy. Note the high color and volume indexes.

Case 120 is probably the first case of sickle-cell anemia in which color, volume and saturation indexes have been calculated. However, we had to use adult white standards, since those for negro children are not known. It seems probable that all the indexes will be normal in sickle-cell anemia if calculated from correct standards.

In the four cases (121 to 124) of acute hemorrhage all the indexes were normal. Case 124 is our only case of acute blood loss in a healthy young man, and is particularly interesting in that records are available before and at intervals after donating 875 c.c. of blood. This loss did not reduce the red cell count or the hemoglobin below the lower limits of normal (no anemia). All blood findings returned to practically their previous level within a month.

Nonanemic Group.—There were a large number of cases originally diagnosed as anemia which could not be used in this paper as true anemia cases; from these we have selected 20 as being particularly instructive (see Table X).

We would define anemia as a decrease in either the red cells or hemoglobin or both below the lower normal limits for persons of the patient's sex and age group. It is possible that in some of these cases there is a relative decrease as compared to the normal level for that particular person (c.f., Case 124) but as previous determinations on these individuals are lacking, it seems only fair to segregate them. This is a precaution which other writers have not observed.

It is noteworthy that the first cases (125 to 130, inclusive) had been erroneously diagnosed as having pernicious anemia. They will be discussed in detail in connection with Table XII.

The next two patients (131 and 132) had been told within a day or so of the date of our study that they had anemia which required treatment. In each instance, the basis for the diagnosis of anemia was a Dare hemoglobin estimation of 65 per cent.

Cases 133 and 134 are examples of the condition called by the Germans "Scheinanämie" or "apparent anemia." They had no symptoms but looked so strikingly pale that it was difficult to believe that anemia was absent.

Cases 135 to 141 are examples of conditions in which anemia sometimes occurs, but which, in these instances, show no anemia. In our experience

*This case is to be reported by Dr. Rush[59].

TABLE X

NO ANEMIA

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
*125	M	47	5.54	110.6	15.26	48.16	0.94	1.06	0.88	Combined system disease.
*126	M	44	4.70	104.0	14.35	42.43	1.04	1.10	0.94	Combined system disease.
*127	F	63	4.40	81.8	11.29	36.02	0.92	0.95	0.96	Hyperplastic exophthalmic goiter.
*128	M	54	5.06	111.5	15.39	42.07	1.03	1.01	1.02	Hypertensive cardiovascular renal disease.
*129	M	42	5.98	134.4	18.55	46.55	1.06	0.95	1.11	Ventral hernia. Appeared anemic. Iron and liver for three months.
**130	F	33	5.59	114.2	15.76	47.04	0.99	0.98	1.01	Chronic cholecystitis. (Thought to be pernicious anemia and ulcer.)
131	M	41	4.95	107.0	14.77	44.36	1.01	1.09	0.93	Normal. Supposed to be anemic.
132	F	18	4.49	95.5	13.18	40.65	1.03	1.05	0.98	Normal. Supposed to be anemic.
133	F	30	4.64	97.8	13.50	43.12	1.02	1.08	0.94	Normal. Appeared anemic.
134	F	21	4.97	84.0	11.59	37.97	0.82	0.89	0.92	Normal. Appeared anemic.
			4.64	102.5	14.15	39.95	1.07	1.00	1.07	Same, three years later.
135	F	16	4.12	101.0	13.94	35.26	1.18	1.00	1.18	Tapeworm. Taenia saginata.
136	M	70	4.74	96.4	13.30	38.23	0.95	0.98	0.97	Hypertensive cardiovascular renal disease.
**137	M	29	5.48	126.6	17.47	49.37	1.08	1.10	0.99	Gastroenteritis, hepatitis, and icterus from poisoning with bootleg liquor.
138	M	23	4.59	88.1	12.15	34.15	0.90	0.91	0.99	Typhoid fever—two weeks.
139	F	19	4.52	84.0	11.59	36.30	0.90	0.93	0.96	Infectious mononucleosis.
**140	M	24	5.71	95.8	13.22	40.11	0.79	0.86	0.92	Pick's disease. Active tuberculous pericarditis.
141	F	26	5.72	88.3	12.18	40.51	0.75	0.82	0.90	Rheumatic mitral stenosis and regurgitation. (Thought to be bacterial endocarditis.)
142	F	20	5.12	84.4	11.65	36.34	0.80	0.83	0.96	Normal with slight chlorotic tendency. Sister of No. 10.
143	M	12	4.96	106.7	14.73	40.87	1.01	1.00	1.00	Normal. Son of No. 71.
144	F	17	5.01	103.5	14.28	40.07	1.00	0.93	1.07	Normal. Daughter of No. 71.

*Previous diagnosis of pernicious anemia.
**Diagnosis confirmed by necropsy.

TABLE XI

PERNICIOUS ANEMIAS CLINICALLY MISDIAGNOSED

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	ORIGINAL DIAGNOSIS
*3	M	24	0.53	16.8	2.32	6.58	1.50	1.53	0.98	Lung abscess with anemia.
10	F	16	1.02	34.7	4.79	14.33	1.64	1.63	1.01	Familial hemolytic icterus.
15	M	44	1.33	40.7	5.62	15.36	1.44	1.41	1.02	Carcinoma of stomach.
21	M	78	1.51	46.4	6.41	17.25	1.44	1.39	1.04	Anemia of unknown type.
*23	F	50	1.77	50.7	7.00	19.58	1.38	1.29	1.07	Anemia (not pernicious).
29	M	56	2.42	64.9	8.95	26.84	1.24	1.36	0.91	Chronic cholecystitis with anemia.
31	F	66	2.76	72.0	9.94	32.50	1.26	1.37	0.92	Chronic cholecystitis with anemia.
32	M	45	2.53	74.0	10.21	32.98	1.37	1.59	0.86	Chronic cholecystitis with anemia.

*Diagnosis confirmed by necropsy.

anemia is the exception in hypertensive cardiovascular renal disease, infectious mononucleosis and in tuberculosis which is uncomplicated by hemorrhage or secondary infection. The absence of anemia in infectious mononucleosis is an important point in differentiating it from acute lymphatic leucemia which it may closely resemble in other respects.

Cases 143 and 144 were examined solely because the mother (Case 71) showed numerous elliptical red cells, but their bloods contained only a few such cells.

Pernicious Anemias Clinically Misdiagnosed.—More than these eight cases (Table XI) might have been included had we not strictly adhered to our rule to discard all those cases in which a reasonably certain diagnosis was not ultimately reached by other methods than a study of the color, volume and saturation index. The color and volume indexes were high in every case at the time when the erroneous diagnosis (see last column of the table) was made. If the criterion of a color or volume index above 1.25 had been used by the clinician, there would not have been this failure of correct diagnosis in 20 per cent of the pernicious anemia cases.

Erroneous Clinical Diagnosis of Pernicious Anemia.—These 13 cases are summarized in Table XII. Note that if reliance had been placed on the color and volume index, question could have arisen only in Case 70 and in that case the indexes do not exceed the highest observed in perfectly healthy persons. Nevertheless, each of these cases was diagnosed pernicious anemia at the time the first recorded studies were made. The correct diagnoses ultimately reached are noted in the last column. Observe that the conditions which are likely to be confused clinically with pernicious anemia are, with the exception of carcinoma of the stomach, not those ordinarily mentioned in texts as difficult to differentiate.

Case 92 was demonstrated twice to senior medical students by well-trained internists as a typical case of pernicious anemia. Following the detection of normal color and volume indexes, positive blood cultures for Streptococcus viridans were secured and a postmortem examination revealed a typical subacute bacterial endocarditis with the rare complication of an abscess in the spleen from which Streptococcus viridans was cultured.

This initial clinical diagnostic error of 13 cases out of a possible 106, or 12 per cent, is to be contrasted with an error of only two cases (98 and 111), or 2 per cent, if both a color and volume index below 1.25 be taken as the criterion for excluding the diagnosis of pernicious anemia. Here again this series might have been increased by less strict adherence to our rule for discarding cases.

DISCUSSION

It is regretted that opportunity did not present for the study of the anemias of sprue, Dibothriocephalus latus infestation, aplasia of the bone marrow, or familial hemolytic icterus. It seems, however, definitely established that the color and volume indexes may be high in some cases of anemia associated with sprue[61] or infestation with Dibothriocephalus latus.[43] It has not, however, been sufficiently emphasized that the pernicious anemia syndrome is the exception rather than the rule in patients harboring the broad tapeworm.[62]

TABLE XII

ERRONEOUS CLINICAL DIAGNOSIS OF PERNICIOUS ANEMIA

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN PER CENT	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	CORRECT DIAGNOSIS
*92	M	38	3.50	75.4	10.40	33.33	1.01	1.16	0.87	Subacute bacterial endocarditis with Streptococcus viridans abscess in spleen and bacteremia. Bleeding hemorrhoids.
42	F	57	2.45	27.0	3.73	14.93	0.53	0.71	0.75	Bleeding hemorrhoids.
*52	M	60	1.96	30.1	4.15	13.78	0.72	0.86	0.84	Carcinoma of stomach.
54	M	43	2.70	33.9	4.68	16.84	0.59	0.76	0.78	Carcinoma of stomach.
*55	M	58	3.26	37.2	5.14	16.67	0.54	0.62	0.86	Carcinoma of stomach.
			3.78	41.7	5.76	21.78	0.52	0.70	0.74	
*70	M	54	1.74	44.0	6.07	17.59	1.18	1.23	0.96	Chronic cholecystitis and chronic fibrous peritonitis.
71	F	39	4.24	48.5	6.69	21.54	0.55	0.59	0.93	Chronic cholecystitis with stones.
			4.10	44.8	6.18	20.59	0.53	0.58	0.90	
*138	F	33	5.59	114.2	15.76	47.04	0.99	0.98	1.01	Chronic cholecystitis. (No anemia.)
125	M	47	5.54	110.6	15.26	48.16	0.94	1.06	0.88	Combined system disease. (No anemia.)
126	M	44	4.70	104.0	14.35	42.43	1.04	1.10	0.94	Combined system disease. (No anemia.)
127	F	63	4.40	81.8	11.29	36.02	0.92	0.95	0.96	Toxic hyperplastic (exophthalmic) goiter. (No anemia.)
128	M	54	5.06	111.5	15.39	42.07	1.03	1.01	1.02	Hypertensive cardiovascular renal disease. (No anemia.)
129	M	42	5.98	134.4	18.55	46.55	1.06	0.95	1.11	Ventral hernia. (No anemia.)

*Diagnosis confirmed by necropsy.

It seems certain that the color, volume and saturation indexes are normal in true aplastic anemia, although we have studied only one case (not included in the series). If Alder's[29] observation that the cell volume (volume index) is normal while the cell diameter is decreased in familial hemolytic icterus, can be substantiated, this will be one disease in which the tedious determination of the average red cell diameter adds to the information secured by the easily determined volume index.

Murphy and Fitzhugh[64] have published data on a considerable number of anemias from which the color, volume and saturation indexes may be calculated. Their results agree quite well with those herein reported. The tendency to a low saturation index in chronic hemorrhage is strikingly demonstrated by calculation of these indexes from the data in their table, although they do not comment on that fact. The few exceptions are probably due to the occasional use of the Dare or Tallqvist hemoglobin methods, although most of their data were secured by the use of an accurate method. We heartily agree with their conclusion that the determination of cell volume is a more simple means of determining the average size of the red cell than is the measurement of the mean cell diameter.

We regret that the time and funds available for this study did not seem to warrant a determination of the total blood and plasma volumes in each case, as this would have undoubtedly contributed further information of value.

Criteria for the differential diagnosis of 28 types of anemia based chiefly on the other laboratory and clinical studies in this series of cases are summarized in a table in another article,[63] together with a discussion of the fundamental causes of anemia.

SUMMARY

The literature on the color, volume and saturation indexes is reviewed. Detailed results, obtained by methods of research accuracy, are reported for hemoglobin, red cell count, cell volume, color index, volume index, and saturation index on 144 cases of anemia and related conditions.

CONCLUSIONS

1. Capps's work on the color and volume index in anemias has never received the credit it deserves.

2. Bönninger (1919) was the first to calculate in essentially its present form the ratio which is now called the saturation index, although Haden (1923) deserves credit for coining this term and for reawakening interest in these indexes in English-speaking countries.

3. The most characteristic change in pernicious anemia is the preponderance of macrocytes in the blood. This is most easily recognized by the volume index determination.

4. The high color index in pernicious anemia is due to the increase in size of the cell, not to increased concentration of hemoglobin within the cell. In other words, the saturation index is normal, and true hyperchromia does not occur either in pernicious anemia or in any other condition so far studied.

5. If either the color or volume index is above 1.25, the patient, with rare exceptions, will prove to have pernicious anemia.

6. Such a high color and volume index constitutes a definite indication for the administration of "liver" therapy.

7. A low saturation index, as was first pointed out by one of us in 1926,[5] strongly suggests that the anemia is due to chronic blood loss, and constitutes a definite indication for a search for the cause of the bleeding, an effort to stop the bleeding, and for the administration of "iron" in large doses.

8. Anemia is rare in patients having malignant tumors without bone marrow metastases, hemorrhage or secondary infection. Hence, the finding of anemia in such patients should suggest that one or more of these complications has occurred.

9. The color, volume, and saturation index determinations are extremely valuable aids in the differential diagnosis of anemias. It is necessary to warn, however, that they may be not only of no assistance, but actually misleading, if they are not determined by accurate methods with controlled technic and if the calculations are not based on the correct normal standards now available, instead of on the obsolete values still included in most texts.*

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CURRENT VIEWS ON THE ORIGIN AND MATURATION OF THE CELLS OF THE BLOOD*

CHARLES A. DOAN, M.D., COLUMBUS, OHIO

MUCH of the accuracy in interpretation of the blood picture in disease is dependent upon an understanding of the underlying mechanism of hemopoiesis. The many technics, fixed, vital and supravital, which have been developed for the study of the cells of the blood, together with data on cell origins and differentiation obtained from embryologic and experimental investigations, now provide at least a working basis for the approach to the dyscrasias involving the hemopoietic system. Quite aside from the primary blood diseases, there are very few pathologic conditions, with which the body has to deal, in which one, or more, of the types of cells represented in the circulating blood is not secondarily involved. That these circulating cellular elements may represent an important and powerful increment in the defense forces of the body is a fact attested by repeated observation. G. Lovell Gulland[1] in his Harveian Oration on the Circulating Fluid "looks forward to the time when the differential count will be more important than the auscultation of the heart," but that day will come in its fullness only when "the knowledge to interpret such data properly" is ours.

The bone marrow throughout extrauterine life is the natural source of erythrocytes, thrombocytes and the three kinds of granulocytes. The phagocytic macrophages (clasmatocytes), while always present in marrow, are quite as regularly and normally found in spleen, liver, and diffuse connective tissues. Their size and ability to arise in situ throughout the body, as the need presents, tend to minimize their appearance in the circulation.[2, 3] Lymphocytes and monocytes, the remaining elements, which utilize the vascular bed as an avenue of distribution, develop in lymph nodes, spleen, and in the more diffuse lymphoid and connective tissues of the body. Hence, hemopoiesis in its broadest sense involves bone marrow, spleen, lymph nodes, connective tissues and the vascular and lymphatic systems.

Several circumstances have contributed to the confusion, which has existed, and which still continues to prevail, among hematologists relative to the origins and relationships of the cells of the blood. It is agreed that all take their first beginning from the mesenchymal cells of the mesodermal layer in the embryo. But thereafter the theories and hypotheses diverge more or less radically, though I would venture to assert that the differences arise more in the interpretation than in opposing objective observations, where experiments have paralleled in materials and methods. In the usual hyperplastic red marrow, where blood cells are developing, it is difficult to distinguish the tissue landmarks, which separate and segregate the units comprising different cell strains. This difficulty

*From the Department of Medical and Surgical Research, the Ohio State University.

has been obviated by certain investigators through a study of less complex areas, either experimentally produced[4, 5, 6] or naturally occurring, as in the zone between red and yellow marrow, when hemopoiesis is extending.[7] Under these conditions erythrogenesis may be observed to occur in foci separate and distinct from myelogenic centers, and it is from analyses of such material that we have learned most about the relative location, distribution and independent origin of erythrocytes and granulocytes. One of the principal difficulties to an unanimous agreement upon these intercellular relationships resides in the fact that all of the cells of the blood continue to arise throughout life as primitive, immature elements incapable of performing their specific functions until after the completion of a definite maturation cycle. Most of the criteria characteristic of the definitive cells, and which form the basis for morphologic differentiation and classification, are elaborated during this maturation period. Hence, the earlier precursors of each strain of cells lack, in direct proportion to their immaturity, clear-cut distinguishing features upon which hematologic investigators can agree. Furthermore, the evidence obtained from tissue culture has tended to emphasize the importance of environment, and of particular food materials, in modifying the morphologic characteristics of cells. This evidence has broadened the basis for the concept of cytologic dedifferentiation, and correspondingly diminished the probability of limited or highly specific potentialities, more particularly as applied to the lymphocyte.[8] It is not strange, therefore, that many students of the subject (Maximow, Downey, Weidenreich, Danchakoff, Ferrata) have concluded that one primitive, multi- or toto-potential lymphocyte, or lymphocyte-like stem cell, the lymphoidocyte of Pappenheim, provides the sole ancestral background for all of the definitive blood cells now recognized. A consideration of this element must, therefore, precede any consideration of the other blood cells.

THE LYMPHOCYTE

The typical lymphocyte of the circulating blood lacks the hemoglobin, the specific granules or the neutral red rosette, which characterize the erythrocyte, the granulocyte and the monocyte, respectively. It, thus, lacks the more readily recognizable criteria of functional maturity. Moreover, the lymphocyte has never been demonstrated to perform any vital function comparable to that of the oxygen-carrying property of red blood cells, or the phagocytic defense or proteolytic enzyme actions of the neutrophilic leucocytes. The appearance of azur granules, or of an occasional neutral red vacuole, in the cytoplasm of the lymphocyte has only served to confuse rather than clarify the issue.

The extensive, and technically perfect, histologic studies of Maximow,[9] including both embryonic and adult tissues, convinced him of the essential hemocytogenic function of the "lymphocyte," which exists solely to be played upon by the various forces of the body, with the corresponding cellular differentiation a direct result of the need of the moment.

The Clarks[10] have studied extensively the cytologic responses of blood and connective tissues in the web of the frog, and more recently in the mammal by means of the ingeniously placed Sandison "window"[11] in the ear of the rabbit. While identifying monocyte and tissue macrophage as different phases of the

same cell type, they have found no evidence of the transformation of the blood lymphocyte into other definitive forms.

The Lewises[12] have studied the changes occurring in hanging drop preparations of blood from many species. They have followed the development of vacuoles in monocytes to the point where it was impossible to distinguish any morphologic difference between these changed cells and the naturally occurring, highly phagocytic macrophages or classmatocytes of the in vivo tissues. "No such obvious transformation of lymphocytes was encountered," however.

Parker and Rhoads[13] studied the cells removed from the circulation in cases of lymphoid leucemia after incubation for various periods of time. They observed that the lymphocytes "often showed some increase in the number and size of their neutral red granules, which in arrangement frequently suggested a rosette. Such a rosette, however, could not be confused in any way with that of a monocyte owing to the shape and color of the granules."

Carrel and Ebeling[14] found that the lymphocytes disappeared in their cultures of hen's blood, and that only the "mononuclears" were transformed into macrophages and fibroblast-like cells.

Cunningham, Sabin and Doan[15] conclude from their experimental studies that a primitive stem cell for the white blood cells may be recognized in bone marrow, lymph nodes, spleen, and the connective tissues. Fixed cells in the reticulum of these organs give rise by mitosis to a free primitive cell, the size of the small lymphocyte. During times of increased demand for myeloid or lymphoid elements these small round cells may be found in increased number in association with the more mature cells of the respective strains. These studies differentiate this primitive stem cell from the lymphocyte, which latter was never observed by these investigators to develop into other definitive forms.

Jordan and his associates have studied hemopoiesis in the frog extensively over a period of years,[16] and they conclude that the differentiation of lymphocytes, originating from reticulum cells, provides the source for the various types of definitive blood cells in this species. Tischtschenko[17] in a recent investigation of hemopoiesis published from His' laboratory in Berlin, concludes that the myeloid and lymphoid elements of the frog's blood "must belong unconditionally to two different hemocytogenic systems." Bloom[18] cultured the lymph from the thoracic duct of the rabbit and found changes occurring which he interpreted as a metamorphosis of both large and small lymphocytes into typical inflammatory mononuclears, the so-called polyblasts of Maximow. Seeman[19] working in Aschoff's Institute of Pathology at the University of Freiburg has found in studying the blood of the white rat that the "real lymphocyte from lymphatic tissue is not capable of transformation into monocytes and histiocytes," and states furthermore, that "the extreme monophyletic school of Maximow places under the category of lymphocytes entirely different forms of cells, which are only separated by supravital staining or biologic experiment."

Applying these two methods suggested by Seeman, Wiseman[20] has recently adduced some very pertinent facts bearing on this question of the identity of the lymphocyte. This investigator first analyzed all of the criteria, which have been described as accompanying maturation in erythrocyte and granulocyte. Quite aside from the elaboration of specific materials in these better understood

strains of cells, which will be described later, certain other significant changes occur. The dense basophilia of the cytoplasm common to all very young blood cells gradually disappears as hemoglobin or specific granules increase. The mitochondria, as revealed by supravital staining with Janus green, rapidly decrease in number with the approach of full maturity, though in the earlier stages of development they are present in large number. The elaboration of chromatin material in the nucleus brings about a very definite change in the density of this structure, from a young vesicular to a mature pyknotic state. With these criteria in mind, a superficial survey of the lymphocytes of the blood revealed differences in nuclear structure, basophilia and mitochondria strikingly similar to those noted in the other blood cells and which could quite readily be submitted to study and critical analysis. Wiseman analyzed these differences first in the normal adult, then in the newborn, and finally under conditions of experimentally induced, lymphoid, hypo- and hyperplasia in rabbits. He has classified the lymphocytes into three groups, according to the criteria cited. In the normal adult human being and the rabbit a relatively stable equilibrium in the proportion of 5 young, to 49 mature, to 46 old lymphocytes is maintained. As would be expected, if the criteria chosen really reflect the relative age of these cells, there was a marked "shift to the left" from this normal Y-M-O formula in the newborn and under conditions of lymph node hyperplasia. That is to say, Wiseman has recognized through these studies a maturation cycle for the lymphocyte of the circulating blood, based upon changing criteria identical with those which accompany the essential transformation of megaloblast to erythrocyte and of myeloblast to neutrophilic leucocyte, with a shifting of the Y-M-O index depending upon the state of activity of the lymphoid tissues. He has thereby established the lymphocyte, it would seem,—this enigma of the hemopoietic system—upon an equal and independent basis of existence with the other blood cells. A very distinctive type of motility for the mature lymphocyte is readily seen in living preparations of blood[21, 22] which contributes further circumstantial evidence, perhaps, toward its independent identity.

It should now begin to be apparent why the interpretations of equally competent and careful investigators have differed so widely up to and including the present time. Superficially, and even with the most meticulous care in analyzing fine cytologic differentiation on a morphologic basis alone, primitive basophilic cells destined to elaborate hemoglobin, or granules, or vacuoles partake of the undifferentiation, which marks to a large degree the entire life cycle of the blood lymphocyte. Only the most discriminating observation, and the submitting of these cells to such biologic experimentation and stimulation as involve an accentuation of their number and activity, can be expected to continue to further our knowledge and understanding of this strain of cells. Our present information, then, justifies both the position taken by those who have contended that "lymphocyte-like" cells may differentiate into other specific definitive cell types, and those who have seen no evidence of transformation in a large proportion of the small "mononuclear" round cells, which exist in blood and tissues. Neither hypothesis may be considered as mutually excluding the other.

Those who have used the supravital and fixed technics extensively together, each for its respective unique contribution to the problem, feel that the early

precursors of the lymphocyte cycle, the lymphoblasts, may be distinguished from the "primitive stem cell"[15] of the white cells, and also from myeloblast and monoblast. The lymphoblast has a relatively deeper basophilia, larger rod-shaped mitochondria and fewer nucleoli (one to two) than do the leucoblasts of the other two strains of white cells. These differences may best be studied as exemplified in the "blast" cells of the acute leucemias, myeliod, lymphoid, and monocytic, where the presence of more mature stages in the respective cell types clearly identifies those with less characteristic criteria. In this problem we have an excellent example of the contribution, which the experimental study of physiologic function, and of changing, rather than static, biologic form, may make to the science of pure morphology, so frequently unappreciative of the aid which may be rendered by these handmaidens.

The implications of the fundamental studies of Wiseman, which are now being applied to the study of the diseases involving the lymph nodes and lymphatic tissues, give promise of a very real advance soon in our understanding of the different pathologic mechanisms underlying the dysfunctions effecting the lymphocytes.

THE GRANULOCYTE

As has been stated already, the principal difference of interpretation with reference to the details of the mechanism of hemocytogenesis abides in the discussion as to just where, if at all, specificity and irreversible dedifferentiation finally begins. Before any specific granules have been elaborated, there may be found in the liver and spleen of the embryo, and in the bone marrow at later stages, extravascular round cells, with a moderately basophilic cytoplasm, many fine, spherical mitochondria, and a vesicular nucleus containing from two to five nucleoli, these cells usually in close association with other extravascular units containing a few scattered granules. Partly because of their extravascular position, in part because of their morphologic characteristics as outlined, and in part through their association with definite myelocytes, these units may be identified as myeloblasts. Though Naegeli, to whom we owe the original concept of the myeloblast, does not limit the term to nongranular cells, it would seem best to reserve this designation for the agranular precursor of the myelocyte. An involved and highly technical nomenclature, together with differing definitions for identical terms employed by the several schools of hematologic investigators, have combined to surround this subject with an unnecessary mystery and complexity. Certain phenomena occur simultaneously and usually reciprocally during the period of maturation of the granulocyte, and to comprehend the underlying facts in this metamorphosis is far more important than to be able to understand all of the terms which have been applied to the many minor morphologic variations which may be found among the myeloid cells, wherever they are observed to be developing.

The primitive, "lymphocyte-like" precursor of the myeloblast may be recognized most readily in relatively hypoplastic areas where myelopoiesis is actively extending. Under such circumstances mitoses may be observed occurring in the fixed cells of the intervascular spaces of the marrow reticulum, situated similarly, topographically, to myeloid foci already functioning. The mitochondrial and basophilic content of the free cells thus formed increase to

a maximum just prior to the appearance of the first few specific granules. From this point on there is a rapid increase in the number of specific granules with a gradual disappearance of the mitochondria and basophilic substance. The only necessity for distinguishing various stages in this maturation period and christening them with names is to facilitate an appraisal of the acuteness of the process in a given case of myelogenous leucemia or severe infection, and to govern therapy. For these purposes the most accurate and dependable single criterion of maturity is the number of granules present in the cytoplasm of a given cell. The chemistry of the granules, basophilia, nuclear criteria, etc., are much more variable with respect to the ultimate criterion of maturity, which is active motility of the cell. Except under conditions of extreme toxicity, when an occasional motile neutrophile may be seen in the peripheral circulation with an incomplete complement of granules, a cytoplasm filled with granules, plus motility, are the essentially constant findings significant of functional maturity in this strain of cells.

But to provide means of statistically expressing the severity of the bone marrow insult or leucemoid hyperplasia in the patient, it has been suggested[23] that the phase of granule elaboration, the myelocyte period, so-called, be arbitrarily divided into three stages: those cells containing ten or less specific granules by actual count may be designated Myelocytes A; when approximately one-half the cytoplasm is filled with specific granules, mitochondria and basophilic material being still plentiful, this adolescence of the cell may be designated as Myelocyte B; just before the nucleus elongates preparatory to the formation of two lobes and before any evidence of motility is apparent, but yet with the cytoplasm filled with granules, we have what may be termed the most mature of the myelocytes, Myelocyte C.

As soon as the granulocyte becomes motile, then, except for the occasional vestige of those cytoplasmic criteria important during the myelocytic maturation, no further changes are noted except in the nuclei, until the final "nonmotile" stage of Sabin,[24] representative of the physiologic death of the cell. Arneth,[25] and later Schilling,[26] and still later many others, have proved conclusively that the number of lobes in the nucleus of the neutrophile is the criterion of age for the mature leucocyte. Early and mild reactions affecting the myeloid function of the bone marrow may be detected by an increase in the percentage of those neutrophilic leucocytes having only two lobes, or no lobing of the nucleus. A still further "shift to the left," and its degree, may be ascertained by partitioning the myelocytes according to the cytoplasmic criteria above mentioned, the study of the whole cycle thus providing data upon which to estimate quite accurately the state of myeloid activity in the bone marrow.[47]

The various degrees of leucopenia, which are now being recognized clinically, including the malignant neutropenia of Schultz[27] may be divided according to whether an actual deficiency of myeloid cells exists in the bone marrow or whether other factors have combined to produce temporarily a lowered count in the blood stream, with the potential source of supply still intact.[28] The rationale of nucleotide therapy[29, 30, 47] in neutropenic conditions must depend upon a very careful differential diagnosis of the underlying mechanism responsible for the finding.

The maturation cycles of eosinophile and basophile vary in no particular from that described for the neutrophile, the earliest granules in these cells showing the special morphologic and staining characteristics so well known in the respective definitive cells.

No longer is it sufficient to know only the total white cell count and the relative proportion of granulocytes, lymphocytes and monocytes present, if the maximum of information is to be secured in the clinical appraisal of the patient. We have already indicated the increased value which may attend the additional qualitative study of the granulocytes and lymphocytes, and it can be said that this general principle is in no wise excepted when it comes to the study of the monocyte.

THE MONOCYTE

Until very recently the nongranular cells of the blood were all classified together as lymphocytes, the transitional or large mononuclear of Ehrlich, or, as it is now more frequently designated, the monocyte, receiving little attention from physicians. When stained with any of the Romanowsky dye combinations, the monocyte of human blood usually shows a myriad of tiny azurophilic granules studded in a background of mottled blue cytoplasm. Because of this finding it was thought originally to represent a transitional stage between lymphocyte and granulocyte. Later, when Michaelis and Wolfe[31] demonstrated so-called azur granules in certain of the lymphocytes, the monocyte was more closely linked in the minds of many with the lymphocyte. Schilling[32] was the first to quite definitely and finally classify it as a separate entity within the group of white blood cells. The studies of the past decade have now made it very clear that many of our most important interpretations in clinical medicine are dependent upon the accurate identification of monocyte and lymphocyte in the differential count.

An appreciation of the morphologic characteristics, physiologic function and pathologic potentialities of the monocyte has followed close upon the development of the supravital staining technic,[24] and the modern experimental and clinical study of tuberculosis.[33] Sabin, Doan and Cunningham[34] described a rather characteristic rosette of neutral red vacuoles surrounded by mitochondria, which are not apparent in the ordinary fixed preparations. The arrangement and behavior of these vacuoles, together with a very distinct surface-film type of motility in living preparations, have served to differentiate sharply the monocyte from the lymphocyte.

That the monocyte is closely related to the "primitive cell" of the connective tissues, similar in all morphologic and other characteristics, to the primitive cells of the lymph node and bone marrow, has been clearly demonstrated. Doan and Sabin[35] noted the appearance of large numbers of monoblasts devoid of vacuoles in the bone marrow of rabbits experimentally infected with bovine tuberculosis. These cells soon developed the typical rosette of neutral red staining vacuoles and were then rapidly transformed into typical mono- and multinucleated epithelioid cells arranged in tubercles. The same sequence of events has been observed in the cytologic proliferation occurring in the various organs and tissues of the body during the development of a generalized tuberculous infection.

Forkner[36] has studied particularly the normal formation of monocytes within the peripheral nodes of normal rabbits, and has described and effectively illustrated, large, young monoblasts, premonocytes or early monocytes showing the beginning development of vacuoles, and the mature monocyte with its fully developed rosette all within one focus, quite as we find myeloid foci with cells in various stages of maturity in the bone marrow.

Hyperplasia of monocytic tissue is thus quite as possible and frequent an occurrence as myeloid or erythroid hyperplasia, and, when it occurs, is reflected quite as quickly and directly, qualitatively as well as quantitatively, in the peripheral circulation. The monocyte-lymphocyte ratio is of distinct prognostic significance in tuberculosis, the quality of the individual cells in each of the categories being equally important. While tuberculo-lipoids have a very strong irritative or stimulative effect upon the monocytes very many other substances also call forth this response in a greater or lesser degree, and many types of general tissue reactions involve the monocytes.

THE TISSUE MACROPHAGE OR CLASMATOCYTE

In the first section of this discussion, it was made apparent that no unanimity of opinion exists at the present time relative to the monocyte-clasmatocyte question. Most of the evidence from tissue culture tends to support the contention that they represent two phases in the life history of the same cell type. While recognizing the occasional highly phagocytic monocyte as approaching very closely, and possibly, rarely, entirely simulating, the tissue macrophage in histologic appearance, nevertheless, the great majority of the phagocytic cells may be very readily separated into two groups in the supravital studies after various experimental procedures. Different pathologic processes strikingly call forth one or the other in rather characteristic predominance, though both are, of course, frequenters of all the organs and tissues.

The large phagocytic cells filled with disintegrating erythrocytes in spleen, lymph nodes, and bone marrow, and the Kupffer cells of the liver sinuses appear to be closely related to endothelium. In the sinuses of lymph nodes, which are draining an area of hemorrhage or necrotic debris, the endothelial cells engulf in situ the foreign material to such an extent that in many places the sinus endothelium will have been entirely denuded and the free, rounded up, highly phagocytic endothelial cells will lie free in the tissues indistinguishable from those in marrow and spleen normally. Whether there is also an extravascular origin for cells with this very highly specialized scavenger activity remains problematical and unsettled up to the present time. Perhaps, we may assume a three-fold source of these tissue macrophages on the basis of the evidence available; from endothelium, from monocytes, and from preexisting clasmatocytes.

Only rarely do these cells find their way into the peripheral capillary circulation[2, 3] probably because of their large size and inelasticity, when highly phagocytic.

THE BLOOD PLATELETS

Both direct and inferential evidence has continued to accumulate during the past quarter of a century, attributing to the magakaryocyte of the bone

marrow the origin of the blood platelets. The unsatisfactory state of the technical procedures for estimating the total number of these bodies is revealed in the multitude of methods, which flood the literature, and the wide range in the figures usually reported. It, thus, becomes important to analyze these essential blood elements qualitatively to supplement and strengthen the weakness inherent in the attempt to determine their quantity.

Apparently these smallest of the formed elements of the blood behave very much like the larger blood cells already discussed. When the platelets are markedly reduced in number under pathologic conditions, there is variation in size with a relative increase in the smaller forms. In addition there is a varying degree of basophilic staining of the cytoplasm with irregular distribution of the tiny granules. When the count is high, or when the platelets are rapidly increasing in number, they tend to be larger and less granular. In the period of rapid increase, which reflects a rapid multiplication of magakaryocytes in the marrow, basophilia is common. Basophilia of the platelet cytoplasm indicates immaturity,[37] as it does in all of the blood cells.

THE ERYTHROCYTE

The erythrocyte is the first of the blood cells to appear in embryonic development. Following the extensive studies of fixed embryonic material by Maximow[38] and Danchakoff,[39] Sabin[40] studied the living chick blastoderm of the second day of incubation, observing the differentiation of the first angioblasts from the primitive mesoderm. These angioblastic nests were then seen by her to give rise to the first blood plasma through liquefaction of some of the central cells. Those on the periphery elongated to form the first vascular endothelium, and the remaining cells comprised the original blood islands of erythroblasts appearing in the area pellucida. Subsequently, Sabin observed the endothelium giving rise to free hemoglobin synthesizing units.

Erythrogenesis ordinarily occurs in the adult in relatively hyperplastic areas of the marrow. It has been necessary, therefore, to analyze relatively hypoplastic states, where myeloid and erythroid foci were widely separated, and not too numerous. This has now been accomplished in both the experimental animal,[41] and in studies of selected human material[7, 42] secured at biopsy or postmortem.

In the first place it was found that there exists in marrow an extensive capillary system,[6, 41] most of which usually is collapsed and nonfunctioning as a patent vascular bed, but which potentially is capable of marked hemopoietic activity. Marked hypertrophy and hyperplasia of the endothelial cells lining these "intersinusoidal capillaries" precedes the appearance of the first cells with hemoglobin. The megaloblasts, while partaking of the relative lack of differential criteria noted in all young blood cells in fixed preparations, still are characterized by a faint blush of hemoglobin, when observed in the living state, and possess a rather distinctive vesicular nucleus with one large nucleolus, very occasionally two. In areas of rapidly extending erythrogenesis, endothelial mitoses are numerous, and the first megaloblasts are to be found within the capillary network, closed to the active circulation. Small isolated islands of erythroblasts develop from these first megaloblasts, and on serial section can be demon-

strated[7, 41] within endothelial lined channels, which communicate with the sinusoids directly. By the gradual elaboration of hemoglobin at the expense of a decreasing basophilia and mitochondrial content, the stage of the normoblast is reached. The main reserve of the red cells in marrow is held under normal conditions at this normoblastic level.[43] Extrusion of the pyknotic nucleus precedes delivery of the mature erythrocyte through the conical opening of the erythrogenic capillary, into the active circulation. Reticulation, or some other manifestation of remaining cytoplasmic basophilia, such as polychromatophilia, is the only evidence of youth, which can be recognized in the mature mammalian erythrocyte. The percentage of reticulocytes per cubic millimeter of blood is a measure of the rate of delivery of these units to the circulation.

Once again recurs the difference in interpretation of evidence, which has marked all of the discussions centering about the origin and maturation of the cells of the blood. Does the erythrocyte take its origin from a multipotential, "lymphocyte" under the stimulus of a favorable environment, or, given the propitious circumstances, must there also be a different stem cell with a more specific capacity for synthesizing hemoglobin? The relationship, which seems to exist between endothelium and erythrogenesis in early embryonic life is the logical point of approach to this problem in the adult. The evidence of endothelial activity, which always accompanies erythropoiesis in the bone marrow of the adult mammal, particularly when taken in conjunction with the data available from embryologic studies, would seem to have more significance than that of a mere fortuitous circumstance. However, it is the opinion of many students of this problem that a free hemoblast, "lymphocyte," located extravascularly in the marrow parenchyma is the precursor of the normoblastic nests of cells, which, when mature, either pass through fenestrations in the vascular endothelium, or if, as most investigators now believe, the vessels of the marrow form a closed circulation, erode temporarily by "growth pressure"[44, 45] an opening sufficient to permit entrance of the erythrocytes into the blood stream.

The perplexing questions concerning the place and mode of origin and the mechanism of delivery of the definitive erythrocytes have no direct bearing at the present time upon the practical clinical implications. The sequence of events in the maturation cycle is quite clear, and this knowledge provides the clinical pathologist with a basis for his analysis of the various syndromes involving the red blood cells, a study which must always precede the institution of rational therapy in the individual case. The hypoplastic and aplastic anemias are the result of deficient megaloblastic differentiation. In pernicious anemia the marrow is hyperplastic, but the megaloblasts for the most part lack the power to mature, to elaborate hemoglobin. All of the many types of secondary anemia involve a deficiency in the ability to utilize, or in the supply of, iron and other elements, essential to hemoglobin formation.

Greater advances have been made during recent years in our understanding and control of the anemias than in the diseases involving primarily the other formed elements of the blood. The increased interest, which is centering about the cells of the blood, as they affect both directly and indirectly the general health of the individual,[46] gives promise of a continually improving therapeutic rationale in our attack upon disease.

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THE SIZE AND HEMOGLOBIN CONTENT OF THE ERYTHROCYTE*

Methods of Determination and Clinical Application

M. M. Wintrobe, M.D., Baltimore, Md.

THE fact that even today relatively few physicians interest themselves in details concerning the size and hemoglobin content of red corpuscles in attempting to differentiate the anemias, is surprising when one finds that some of the most important physical characteristics of the erythrocyte and their variation in disease have been known almost since the first days of the microscopic study of blood. Welcker, who with Vierordt recorded the earliest erythrocyte counts, determined the volume of the red corpuscle with the aid of plaster models. He found (1864) that the blood corpuscles in a case of chlorosis were smaller than normal. Johannes Duncan about the same time (1867) recognized the possibility of variation in size and hemoglobin content of red corpuscles and Sørensen (1876), Ehrlich (1880), and Laache (1883), noted the increase in the size of the cells in Addison-Biermer (pernicious) anemia. Hayem (1878) measured the diameter of red corpuscles, calculated their mean volume from these measurements, and devised the color index.

There can be little doubt that these determinations with the exception of the color index, failed to arouse the enthusiasm of the general medical public as much because of technical difficulties as on account of general ignorance concerning the value of such examinations. The earlier hematocrits, such as those of Hedin (1890) and Daland (1891), could not be depended on for accurate and consistent information and even instruments developed more recently, such as the Van Allen hematocrit (1925), have by no means satisfied critical workers. Although the determination of the average amount of hemoglobin in the red corpuscles (color index) has been very commonly carried out, the notorious inaccuracy of hemoglobin estimation, so general even today, has brought this constant into considerable disrepute. The introduction of accurate methods, such as those for the measurement of cell diameter utilized by Price-Jones[30] and others,[15, 35, 2] has likewise not been followed by the universal enthusiasm which the information derived may deserve, because of the time-consuming and tedious nature of these studies. There is little question that general interest in the study of the size and hemoglobin content of the erythrocyte, in spite of proof of the importance of these observations, can only be aroused by the presentation of simple and accurate methods.

METHODS

No attempt will be made in this paper to present completely all the methods which are available for the determination of the size and hemoglobin content of the red corpuscles. Instead, it is proposed to mention only briefly those methods

*From the Medical Clinic, Johns Hopkins University.

which are of historical importance or are of relatively little value and to describe fully those which in the writer's own experience are most useful from the standpoint of simplicity and accuracy.

Measurement of Erythrocyte Diameter.—Although the diameter of red corpuscles was measured in the very early days of hematology, it was not until ten years after the first of the painstaking studies of Price-Jones was published (1910) that considerable interest was aroused in these determinations. Price-Jones projected the images of the red corpuscles on a sheet of paper, and after outlining in pencil the cells in a thin portion of the smear, measured their long and short diameters. The magnification of the cell images being known, it was possible to calculate the actual size of each cell. The results of the measurement of five hundred cells were plotted in the form of a curve which has come to be known as the "Price-Jones curve." The method of Price-Jones, although quite accurate, is obviously too time-consuming for clinical laboratory work. Various modifications have been recommended, the simplest being the following:

A micrometer disc of glass marked with a scale, the smallest division of which equals approximately one micron, is placed in the eyepiece of the microscope. Since tube length and lens differ in various microscopes, it is necessary to calibrate the micrometer disc for the tube length and lenses employed. This is very easily done with the aid of a micrometer slide specially made for this purpose, or a hemocytometer may be used instead. The micrometer disc having been placed in the eyepiece of the microscope, the hemocytometer is focused and adjusted in such a way that the divisions of the micrometer disc are superimposed over one of the smallest divisions of the hemocytometer. Since the latter are exactly fifty microns to the side, it is simple enough to determine how many of the micrometer divisions make up fifty microns, and from this to calculate the width of one division.

Fresh blood or fixed and stained smears may be employed, but it is important to bear in mind that a uniform technic must be followed. Fixing and drying of red corpuscles cause shrinkage in their size.[30, 27] Smears should be well stained and thin. There is some variation in the values reported for normal mean erythrocyte diameter (Price-Jones, 7.2 μ, Grosh and Stifel, 7.4 μ, Bell et al., 7.7 μ, Wiechmann and Schurmeyer 7.9 μ). The cause of these differences is not clear but since they may be due to slight differences in technic, it is advisable to establish the normal for one's own working conditions by examining several specimens of normal blood before carrying out any series of determinations in disease.

The actual measurement of the cells is carried out by bringing the erythrocytes in a thin portion of the preparation under the scale of the micrometer disc. The maximum and minimum diameters of each cell may be measured and the mean of these taken as the diameter of the erythrocyte, or only one diameter of all round cells which appear under the scale may be recorded. It is obvious that greater accuracy is secured by measuring two diameters of each of a large number of cells. Usually several hundred red corpuscles are examined in this way. The mean diameter of all the cells is calculated, but it is also important to note the degree of scatter (the proportion of microcytes and macrocytes) for,

as Price-Jones has emphasized, the scatter may be such that the mean cell diameter is normal or nearly normal. Scatter is best demonstrated by graphically recording the number of erythrocytes of each size encountered (Price-Jones curve).

Comparative Value of Methods for Determination of Cell Size.—The measurement of cell diameter gives quantitative expression for what one sees on examining a blood smear. This information is very valuable but from a practial standpoint must be measured in terms of the time and effort required and the possibility of deriving similar information by simpler methods. Various instruments have been devised by means of which cell diameter may be measured much more easily than by the technic above described. These methods (diffraction methods of Pijper, Millar, eriometer of Emmons, halometer of Eve) are based on the principle that circular, concentric spectra, the character and size of which depend on the size and shape of the red corpuscles, are produced when parallel light rays are passed through a blood smear placed in front of a convex lens. The very important disadvantage of all these methods is that the size of the corpuscles is measured in mass and only mean diameters are thereby determined. Variability cannot be accurately measured. As Price-Jones himself admits, variations in cell diameter are relatively so small and of such variety in disease, that mean cell diameters may often not be significantly altered from the normal. Of thirty-two determinations in 14 cases of pernicious anemia and 5 cases of sprue, I found mean cell diameter greater than the maximum normal in only 15 instances and even in 6 of these the values were little greater than the normal.[40] On the other hand, Haden[16] and I[40] have shown that it is possible to demonstrate striking differences in mean values when the average volume of the red corpuscles is determined, for, in measuring volume small variations in size in all dimensions are taken into account and thereby the differences are magnified. For this reason as well as from the standpoint of accuracy, the measurement of mean cell diameter by one of the diffraction methods is inferior to the determination of mean cell volume by the method to be described. With regard to the measurement of individual cell diameter as is carried out by means of the micrometer disc, no criticism can be offered except that which derives from the tedious and time-consuming nature of the procedure. However, the latter objection is most important from the standpoint of general practicability. In my own experience, the simple examination of a blood smear together with the measurement of mean corpuscular volume has afforded considerably more useful and accurate information at the expense of much less effort than is involved in measuring the diameter of several hundred red corpuscles. The hematocrit method to be described possesses the additional advantage that, in combination with hemoglobin determinations, valuable information concerning the concentration of hemoglobin in the red corpuscles may be gained which cannot be derived in any other way.

Measurement of Corpuscular Volume.—There is no practical method by means of which the volume of individual red corpuscles may be determined. It is only possible to determine the mean corpuscular volume of a substantial number of cells. This is done by determining the volume of packed red corpuscles as well as the number of cells in a given quantity of blood and from these values

the volume of the average erythrocyte is calculated. The volume of packed red cells may be determined by centrifuging a quantity of blood to which a suitable anticoagulant has been added. Centrifugation is carried out until no further packing occurs.

Hematocrits are available which require only such small quantities of blood as may be obtained by finger puncture,[1, 32] but since greater accuracy is afforded by the use of larger quantities of blood, venipuncture is recommended. When care is taken to avoid congestion of the arm from tourniquet pressure, no difference is found between venous and capillary blood. It is our custom to prepare the needle and syringe, clean the arm for venipuncture, and fasten the tourniquet just prior to making the puncture. When this routine has been followed, blood counts on capillary and venous blood have been found to agree exactly. Venous blood so obtained may be used not only for hematocrit determinations but also for red cell counts, hemoglobin, leucocyte, platelet, reticulocyte, and other hematologic procedures. There is the additional advantage that counts may be checked without the necessity of troubling the patient more than once.

Heparin is the ideal anticoagulant, since it does not affect the size of the red corpuscles but it cannot always be depended upon unless the extremely expensive, purified material is employed. The use of an isotonic anticoagulant, such as 1.4 per cent sodium oxalate solution, has the obvious disadvantage that the blood is diluted and a source of error is introduced not only in hematocrit determinations but also in any blood counts which it may be necessary to carry out. For these reasons, dry potassium oxalate in minimal amounts, namely, 10 mg. per 5 c.c. of blood, is preferred. This quantity is easily measured by running 0.5 c.c. of 2 per cent potassium oxalate solution from a burette into a vial or small bottle and allowing the water to evaporate. In the proportion of 2 mg. per c.c. of blood, solid potassium oxalate, does not influence red cell, leucocyte, platelet, or other counts but causes a shrinkage of 8.2 per cent in the volume of the red corpuscles as compared with their volume in heparinized blood.*

For the hematocrit, I have found most useful a glass tube of about 2.5 mm. uniform inside bore and flat inside bottom. On the sides of this instrument,[36] † a centimeter-millimeter scale 10 cm. in length is etched. About 0.7 c.c. of blood is required. The hematocrit is easily filled by means of a capillary pipette. When the leucocyte count is high, it is advisable to allow the filled hematocrit to stand for an hour or longer before centrifugation, in order to permit separation of the red and white corpuscles. Centrifugation at 3,000 revolutions per minute for a half hour is necessary to secure complete packing. It is not necessary, however, to determine the speed of the centrifuge for, since the object of centrifugation is to secure complete packing, it is simple enough to determine the time necessary for any instrument even though its speed is not known.

In order to deal with values which may be readily visualized, it is preferable to calculate the *mean corpuscular volume* in cubic microns. This is done by di-

*The volume of packed red cells, as determined from blood to which has been added solid potassium oxalate in the proportion of 10 mg. per 5 c.c. of blood, should be multiplied by the factor 1.09 in order to correct for shrinkage.

†The hematocrit may be obtained from Arthur H. Thomas Co., Philadelphia, or Will Corporation, Rochester, N. Y.

viding the volume of packed red cells, expressed in cubic centimeters per 1,000 c.c. of blood by the number of red cells expressed in millions per cubic millimeter. The result gives the average volume of the red corpuscles in the sample of blood in cubic microns. Thus, for a sample which contains five million red corpuscles per c. mm. and 42.5 c.c. of packed red cells per 100 c.c. of blood, the mean corpuscular volume is $425 \div 5.0 = 85$ c. μ.

Objections have been raised against this method of determining corpuscular volume. Some observers have thought that by centrifuging, fluid is forced out of the blood corpuscles, thus making their volume smaller. This effect is unlikely because the volume of the blood corpuscles is determined by osmotic forces which are so great that the force with which they are centrifuged down is negligible in comparison. That it does not take place is shown by the fact that the mean corpuscular volume of normal blood as determined by the hematocrit method here described, agrees almost exactly with the value obtained by Ponder and Saslow[29] by an accurate (but laborious) colorimetric method.

From a detailed study of methods of measurement of red cell volume, Ponder and Saslow[29] concluded that the hematocrit method is inaccurate. They used as hematocrits capillary tubes 100 mm. long and centrifuged three sets of these tubes at speeds of 1,000, 4,000, and 14,000 r.p.m. Spinning was carried out to the attaining of constant volume or Koeppe's criterion. They could find no consistent agreement with the results of volume determination obtained by their colorimetric method. It seems likely, however, that the hematocrit technic employed by these investigators is at fault rather than the hematocrit method as a whole, for the values for mean corpuscular volume obtained by the method described by the writer not only agree almost exactly with those of Ponder and Saslow as determined by what they consider a much more accurate method, but the same results are consistently obtained. In Table I will be found the results of four experiments which were carried out to determine the probable error of

TABLE I

PROBABLE ERROR OF DETERMINATION OF VOLUME AND HEMOGLOBIN CONTENT OF RED CORPUSCLES

EXP. NO.	MEAN CORPUSCULAR VOLUME		MEAN CORPUSCULAR HBG.		MEAN CORPUSCULAR HBG. CONCENTRATION	
	STANDARD DEVIATION	COEFFICIENT OF VARIATION	STANDARD DEVIATION	COEFFICIENT OF VARIATION	STANDARD DEVIATION	COEFFICIENT OF VARIATION
1	1.04 c. $\mu \pm 0.25$	1.08%	0.33 $\gamma\gamma \pm 0.07$	0.97%	0.33% ± 0.08	0.93%
2	0.48 c. $\mu \pm 0.11$	0.65%	0.43 $\gamma\gamma \pm 0.09$	2.08%	0.23% ± 0.06	0.82%
3	2.02 c. $\mu \pm 0.48$	1.63%	0.52 $\gamma\gamma \pm 0.13$	1.33%	0.23% ± 0.05	0.72%
4	0.03 c. $\mu \pm 0.01$	0.04%	0.02 $\gamma\gamma \pm 0.01$	0.13%	0.37% ± 0.08	1.46%

the methods followed in our laboratory. In each experiment the following procedure was repeated five times on the same sample of venous blood: (1) two red cell counts were carefully made, using two dilutions (the average of the two was employed in the calculation); (2) the hemoglobin was determined by means of a Newcomer hemoglobinometer which had been restandardized by the Van Slyke method, and (3) the volume of packed red cells was determined, using

a special hematocrit[36] and an International Centrifuge, (Size 1, type SB, head 9 cm.) rotated at 3,000 r.p.m. for thirty minutes. The small error clearly demonstrates the reliability of the results.

Determination of the Hemoglobin Content of the Erythrocyte.—If in addition to determining the number of red cells and the volume of packed erythrocytes in the sample, the hemoglobin be determined as well, it is possible by simple calculation to derive valuable information concerning the hemoglobin content of the red corpuscles. This is an important advantage of the technic here described. As in the case of the red cell counts and hematocrit determinations, accuracy is of course fundamental. We employ a Newcomer hemoglobinometer which has been restandardized by the Van Slyke method. Hemoglobin is determined in grams per 100 c.c. of blood.

The *mean corpuscular hemoglobin* or average amount, by weight, of hemoglobin in the red cells is determined by dividing the amount of hemoglobin, expressed in grams per 1,000 c.c. of blood by the number of red cells, expressed in millions per cubic millimeter. The result gives the average weight of hemoglobin in the cells in micromicrograms.*

The *mean corpuscular hemoglobin concentration* or average concentration or saturation of the red corpuscles with hemoglobin, is determined by dividing the amount of hemoglobin, expressed in grams per 100 c.c. of blood, by the volume of packed red cells, expressed in cubic centimeters per 100 c.c. of blood, and multiplying the result by 100. Mean corpuscular hemoglobin concentration is expressed in per cent. It will be noted that its calculation is similar to the calculation of the strength of any solution. Although the implied presumption that hemoglobin is contained in the red cell in the form of a solution is probably incorrect, the determination of this relationship of amount of hemoglobin to size of cell is nevertheless very valuable, as will be shown later.

TABLE II

METHOD OF CALCULATION OF CORPUSCULAR CONSTANTS

MEAN CORPUSCULAR VOLUME (C.V.) in cubic microns (c. μ)	=	$\frac{\text{Volume of packed red cells (in c.c. per 1000 c.c. blood)}}{\text{R.B.C. (in millions per c. mm.)}}$
MEAN CORPUSCULAR HEMOGLOBIN (C.H.) in micromicrograms ($\gamma\gamma$)	=	$\frac{\text{Hemoglobin (in gm. per 1000 c.c. blood)}}{\text{R.B.C. (in millions per c. mm.)}}$
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (C.C.) in per cent (%)	=	$\frac{\text{Hemoglobin (gm. per 100 c.c. blood)}}{\text{Volume packed R.B.C. (c.c. per 100 c.c. blood)}} \times 100$

The method of calculation may be illustrated by the following:

A sample of blood contains 5.0 million red blood cells per cubic millimeter. 14.5 gram of hemoglobin per 100 c.c. of blood, and 42.5 c.c. of packed red cells per 100 c.c. of blood. Then

*A micromicrogram is the millionth, millionth part of a gram or gram $\times 10^{-12}$ and is abbreviated $\gamma\gamma$.

$$\text{mean corpuscular volume (C.V.) is } \frac{425}{5.0} = 85 \text{ c. } \mu$$

$$\text{mean corpuscular hemoglobin (C.H.) is } \frac{145}{5.0} = 29 \ \gamma\gamma$$

$$\text{mean corpuscular hemoglobin concentration (C.C.) is } \frac{14.5}{42.5} \times 100 = 34\%$$

It will be evident that these calculations are similar to those used for the calculation of the volume, color, and saturation indexes, respectively, differing from the latter only in that absolute instead of relative values are employed. This method is preferable to the calculation of the indexes for the following reasons:

1. The number of red cells and volume of packed red cells for any sample of blood are always determined in absolute terms. In order to calculate the indexes, it is necessary to convert these values into terms of per cent of normal. This is not required for the calculation of the constants here recommended. Again, although hemoglobin is recorded in per cent by many, there is a growing number of critical physicians who appreciate that it is more accurate to express hemoglobin directly in grams for the reason that the use of widely differing standards of normal is thereby avoided. Hemoglobin values expressed in absolute terms (grams) require no conversion into per cent for the calculation of the corpuscular constants.

2. In order to convert the red cell count, hemoglobin and volume of packed red cells to terms of per cent of normal, it is necessary to adopt standards of normal. That such standards must necessarily be arbitrary and erroneous is obvious when one considers the wide fluctuations in normal values associated with differences in sex, age, and possibly also geographical location.[37, 31]

3. Whereas the indexes express only relative values, the corpuscular constants afford absolute information concerning the erythrocyte and permit the visualization of the physical state of the red cell.

CLINICAL APPLICATION

Normal values for the size and hemoglobin content of the erythrocyte are recorded in Table III. The values for cell diameter must be considered as somewhat approximate for, as already mentioned, there are slight differences in the

TABLE III

NORMAL VALUES FOR SIZE AND HEMOGLOBIN CONTENT OF ERYTHROCYTES

	AVERAGE	MINIMUM	MAXIMUM
Mean Corpuscular Volume (C.V.) in cubic microns	87	80	94
Mean Corpuscular Hemoglobin (C.H.) in micromicrograms	29.5	27	32
Mean Corpuscular Hemoglobin Concentration (C.C.) in per cent	35	33	38
Mean Corpuscular Diameter in microns	7.5	6.7	8.0

diameter of the red cell as recorded by various observers. No significant differences in the size or hemoglobin content of the red cells in regard to sex have been noted, but it is now well known that the cells of the newborn are larger than those of adults.[37] Price-Jones[30, 35] and others have observed some increase in the diameter of red corpuscles during the day and as the result of violent exercise. However, neither Dryerre et al.[6] nor Ponder and Saslow[28] have been able to confirm these results, nor have Haden[16] or I (unpublished data) observed similar changes in the volume of the red cell. Likewise, I could find no relation between the size and hemoglobin content of the red corpuscle and the biologic reference—frame represented by body-weight, stature or surface area of men and women.[39] In general, the erythrocyte may be regarded as being remarkably constant in its physical characters and an index of an internal environment regulated to great constancy in the normal individual. This is in striking contrast to the variations observed in disease.

Although Welcker (1864) observed that in chlorosis the red corpuscles are smaller than normal and Sørensen (1876) pointed out that in Addison-Biermer anemia they are abnormally large, it remained for Capps in 1903, to stress the fact that significant alterations in the size of erythrocytes occur in anemia and that these changes are the result of variations in the growth, development, and destruction of the cells. In 1910, Larrabee made similar observations but until the last few years interest in this subject has been only spasmodic.[3, 12, 13, 14, 16]

In association with certain diseases there arise differences in the size and hemoglobin content of red corpuscles, the recognition of which is not only of assistance in diagnosis, but through more accurate differentiation gives important information concerning prognosis and therapy. Not only is it possible by the special hematologic methods here described to recognize pernicious anemia and the other macrocytic anemias but there may readily be distinguished a type of anemia which has hitherto rarely been differentiated from the large group of so-called "secondary" anemias.

It is possible on the basis of differences in the size and hemoglobin content of the erythrocyte to distinguish 4 types of anemia.[38] These are:

1. Macrocytic anemias, characterized by an increase in the mean volume and hemoglobin content of the erythrocytes and represented chiefly by pernicious anemia and many cases of sprue.

2. Normocytic anemias, which are distinguished by the presence of red cells of normal size and hemoglobin content. This group includes cases of anemia resulting from acute blood loss, hemolytic anemias (malaria), and aplastic anemias.

3. Simple microcytic anemias, which are characterized by a moderate reduction in the size of the red cells with no or little reduction in their hemoglobin concentration. In this group are found the majority of the anemias associated with chronic infections and toxic processes.

4. Hypochromic microcytic anemias. In this type of anemia there may be little or no reduction in the red cell count. Nevertheless the red corpuscles are found to be very small and are poorly filled with hemoglobin.

The determination of the size and hemoglobin content of erythrocytes is chiefly important in that it makes possible the ready differentiation of the macrocytic and the hypochromic microcytic anemias from the other two types.

Macrocytic Anemias.—For many years physicians have largely depended on the color index for the recognition of macrocytic anemias. It is common knowledge that in pernicious anemias the color index is greater than 1. It is less generally appreciated that this does not mean that the red cells are hyperchromic or supersaturated with hemoglobin, an erroneous conception to which the use of the color index has led. By the calculation of the size and hemoglobin content of the erythrocyte in absolute terms, a clear conception of the state of the red corpuscles in this disease may be gained. These calculations indicate that the average red cell in pernicious anemia contains a greater amount of hemoglobin than is normal (mean corpuscular hemoglobin is high) but this increase in hemoglobin content is no greater than the increase in size so that the concentration of hemoglobin in these cells (mean corpuscular hemoglobin concentration) is normal and may even sometimes be lower than normal.

The mean corpuscular volume is consistently found to be high in pernicious anemia in relapse. Of 56 cases examined at various stages of relapse, and incomplete remission the mean corpuscular volume was greater than 95 c. μ in 50, and greater than 90 c. μ in 54. Of 37 of these cases in which the red cell count was less than three million, mean corpuscular volume was greater than 100 c. μ in 30, between 95 and 100 c. μ in 2, and between 90 and 95 c. μ in 3. The mean corpuscular volume was lower than 90 c. μ in 2 cases. In one of these transfusion had just been carried out. Subsequent determinations in the other case showed significantly high values. In no case was the mean corpuscular volume lower than normal. The values for mean corpuscular volume ranged as high as 164 c. μ, but usually were found between 100 and 125 c. μ. Although frequently the volume of the cells was greater in those cases in which the anemia was most severe this was by no means always true. The variations in corpuscular volume seem to be related to the nature and extent of red cell formation and destruction, and especially to the reticulocyte response (unpublished data).

One of the most important uses of the determination of mean corpuscular volume is in the recognition of cases of pernicious anemia in which the anemia is slight or moderate in degree. It is well known that at such a stage there may be considerable difficulty in diagnosis, especially in those cases in which neurologic manifestations are present, when confusion with other diseases is not unusual. When the red cell count is greater than three million, the blood smear may not be of much assistance in diagnosis and measurement of the diameter of the red corpuscles may afford results which must be interpreted as normal or so nearly normal that little help in diagnosis is gained. Here the measurement of mean corpuscular volume by taking into account changes in all dimensions and thereby magnifying many small differences, finds one of its most important uses. Haden has pointed out that an increase of 1 micron (13 per cent) in the diameter of a red cell is associated with an increase of 44 per cent in its volume. In Table IV are recorded the first blood examinations in 19 cases which were subsequently proved to be pernicious anemia and in which the red cell count was greater than three million. It is interesting to observe that the magnitude of the corpuscular volume was correlated with the proportion of the red cell count to the normal value for the sex of the patient rather than to the actual value of the count. The cases in which the red cell count was from three to four

TABLE IV

SIZE AND HEMOGLOBIN CONTENT OF RED CORPUSCLES IN CASES OF PERNICIOUS ANEMIA WITH MODERATE OR LITTLE ANEMIA

SEX	R.B.C.		C.V.	C.H.	C.C.
	NO. X 10^6	% OF NORMAL	c. μ	$\gamma\gamma$	%
F	3.21	62	109	38	35
M	3.79	69	107	34	31
M	3.89	71	106	36	34
F	3.57	75	109	40	36
M	4.11	75	103	37	36
F	3.60	75	98	34	35
F	3.66	77	92	32	35
M	4.37	79	100	39	39
M	4.48	81	98	32	33
M	4.54	83	88	32	36
F	4.02	84	84	30	35
F	4.03	84	96	32	33
F	4.17	87	98	34	34
F	4.22	88	98	33	34
F	4.23	89	90	33	37
F	4.24	89	86	29	34
F	4.28	89	94	29	34
F	4.31	90	95	34	36
F	4.48	94	85	32	38

million in females and from three to four and a half million in males, may be considered as representing about the same degree of anemia. Nine cases occur in this group. In 6 the mean corpuscular volume was 100 c. μ or higher and therefore strikingly greater than normal. In 2 cases it was 98 c. μ and still distinctly above normal and in only 1 case was the mean volume at the upper limit of normal values. Again, even when the anemia was very slight (red cell count over four million in females and over four and a half million in males) the mean corpuscular volume was 94 c. μ or greater in 5 of 10 cases. It should be borne in mind that the values recorded represent only the first determinations in the cases cited, and were carried out for the purpose of differential diagnosis. In most instances corpuscular volume determinations were frequently repeated and all these subsequent observations showed results consistent with the statements above made. Fig. 1 illustrates the consistently high corpuscular volume in 3 of these cases observed over a period of three and a half months.

It must, of course, be borne in mind that the finding of a mean corpuscular volume greater than normal does not in itself justify a diagnosis of pernicious anemia. Such a finding simply classes the anemia as being of the macrocytic type. This form of anemia is also characteristic of sprue. Macrocytic anemia is frequently stated, and rarely found, to occur in association with carcinoma, especially of the gastrointestinal tract, pregnancy and syphilis, and is often associated with Dibothryocephalus infestation. I have also encountered several instances of macrocytic anemia in association with certain cases of diarrhea of obscure etiology, disease of the liver, aplastic anemia, and anemia of acute blood loss. Such findings, however, are unusual.

In response to treatment with liver and the pernicious anemia liver extracts, more and more red corpuscles of normal size appear and eventually when

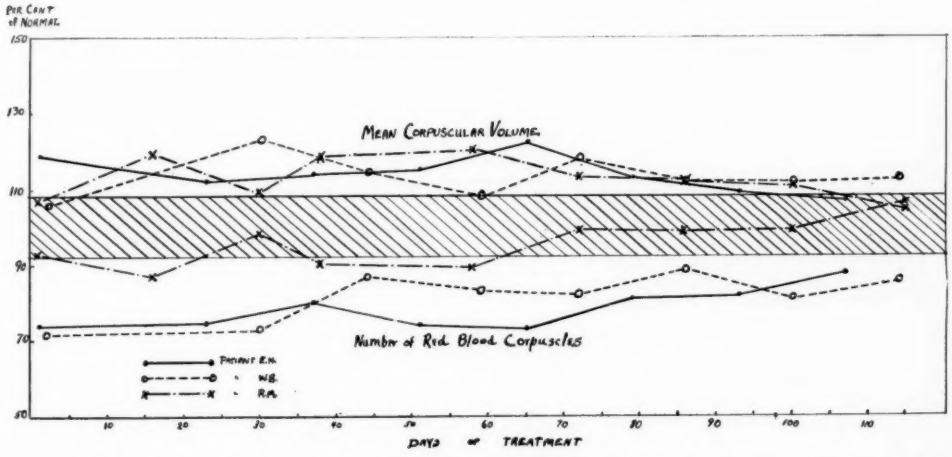

Fig. 1.—Three cases of pernicious anemia observed over a course of three and a half months demonstrating increased mean corpuscular volume in spite of slight degree of anemia. Both mean corpuscular volume and red cell count are represented in proportion to the normal. The shaded area represents the range of normal.

the blood count has reached normal, normal or high normal values are encountered for mean corpuscular volume. This is true for sprue as well as for pernicious anemia.[40]

Alterations in mean corpuscular hemoglobin are similar to those described for mean corpuscular volume. They may, however, be of less magnitude. For this reason, determination of mean corpuscular hemoglobin may be of less value than the calculation of mean corpuscular volume. Furthermore, volume determinations are less open to error since the accuracy of the hematocrit is much greater than that of the majority of hemoglobin methods.

Hypochromic Microcytic Anemias.—In most types of anemia mean corpuscular hemoglobin concentration remains normal or at least little reduced. This is as much true of the simple microcytic forms associated with chronic, toxic, and infectious diseases as of the macrocytic anemias. There is a group of anemias, however, in which mean corpuscular hemoglobin concentration is significantly reduced. This group is also characterized by microcytosis and I have therefore suggested the term "hypochromic microcytic" anemia to dis-

tinguish this group from the anemias in which there is microcytosis without hypochromia (simple microcytic anemia).[38]

It is characteristic of this group to find a low mean corpuscular volume (70 to 55 c. μ). Mean corpuscular hemoglobin is, however, more than proportionately reduced (20 to 14 $\gamma\gamma$) so that the mean corpuscular hemoglobin concentration is distinctly low (29 to 21 per cent). This is in striking contrast to the findings in other types of anemia (Table V). Measurement of the diameter of

TABLE V

SIZE AND HEMOGLOBIN CONTENT OF ERYTHROCYTES IN VARIOUS TYPES OF ANEMIAS

TYPE OF ANEMIA	MEAN CORP. VOLUME c. μ	MEAN CORP. HBG. $\gamma\gamma$	MEAN CORP. HBG. CONCENTRATION %	MEAN CELL DIAMETER μ
Macrocytic	95-160	30-52	31-38	7.5-9.6
Normocytic	80- 94	27-32	33-38	6.7-8.0
Simple Microcytic	72- 79	22-26	31-38	6.5-8.5
Hypochromic Microcytic	50- 71	14-21	21-29	5.8-7.5

the erythrocytes is of less value for, although the Price-Jones curve shows a broadened base, the mean diameter is usually little reduced and may even be within normal limits.[15, 23]

Microcytosis with hypochromia commonly occurs as the results of chronic blood loss and is characteristic of the achlorhydric anemia described by Faber,[11] Witts,[42] Kaznelson,[20] and others.[23] Hypochromic microcytic anemia is not always associated with achlorhydria, however, but is common in persons who have partaken of a diet defective in hemoglobin building substances over a long period of time. This type of anemia probably occurs whenever there is a lack of hemoglobiniferous substances in the diet, when these substances are improperly absorbed or utilized, or when there is excessive drain on the hemoglobin content of the body as in chronic blood loss.

It is in this type of anemia that large doses of iron are so spectacularly effective. Under such treatment more and more cells of normal size and hemoglobin content appear, the mean corpuscular volume and mean corpuscular hemoglobin concentration gradually rise, and finally normal values are attained. Pernicious anemia liver extract is valueless in these cases and even whole liver seems to have little effect. Here again, then, the determination of the size and hemoglobin content of the red corpuscles is of value, for it not only facilitates diagnosis but affords information which is of great value in indicating the appropriate type of therapy.

CONCLUSIONS

1. The differentiation of pernicious and other forms of macrocytic anemia and of anemias characterized by microcytosis and hypochromia from other forms of anemia is not only of academic and diagnostic interest but is essential in the guidance of appropriate treatment.

2. Such differentiation is best made, from the standpoint of simplicity and accuracy, by the calculation of the volume and hemoglobin content of the red

corpuscles. These calculations, when combined with the simple examination of the blood smear, yield more information at the expense of much less effort than is entailed in the measurement of the diameter of the red corpuscles.

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THE SICKLE CELL PHENOMENON*

I. The Rate of Sickling in Moist Preparations

L. W. Diggs, M.D., Memphis, Tenn.

ATTENTION was first directed to the phenomenon of sickling in 1910 when Herrick[1] reported the presence of "peculiar, elongated and sickle-shaped red blood corpuscles" in both fixed and moist preparations from a case of severe anemia. Emmel[2] in 1917 observed that there was an increase in the number of sickled cells when the blood from a case of sickle cell anemia was left standing in sealed coverslip preparations. He also demonstrated the delayed appearance of sickled erythrocytes in the blood of the nonanemic father of the active case. Huck[3] in 1924 noted that the sickled cells assumed a spherical form in from three days to six weeks. Since that time, numerous experimental observations have been made on the sickle cell phenomenon, most of which have been based on the behavior of red cells in whole blood or in various solutions when sealed under a coverslip. A review of the now rather extensive literature reveals that many figures have been given for the percentage of sickled cells appearing in a given case in a given time, but no systematic study has been reported as to the rate of sickling over the entire course of the process. There has been disagreement as to the comparative speed of sickling in sickle cell anemia cases and in cases showing the capacity for sickling under suitable conditions, but without the characteristic hemolytic anemia. The reliability of moist preparations as a method for further experimental work has yet to be established.

In this article, the observations and discussion will be limited to the rate of sickling in moist preparations, to a comparison of the rates in different clinical groups, and to an analysis of some of the sources of error in the method. The reader is referred to recent comprehensive reviews of the literature by Graham and McCarty,[4] Steinberg,[5] and Yater and Mollari[6] for a discussion of other phases of the problem, and for a complete bibliography.

METHOD

Moist preparations were made by taking a drop of blood from a finger puncture on a new alcohol cleaned, No. 1 coverslip, inverting on a new, alcohol cleaned slide, and allowing the drop to spread without pressure except for the weight of the cover glass. The edges of the coverslip were rimmed with vaseline, using a small brush. The preparations were examined immediately, at two- to four-hour intervals during the first twelve hours, and at longer intervals thereafter. The number of readings are indicated by the points on the charts. The preparations were all kept at room temperature. Those which showed evidences of drying at the edge of the drop were interpreted as having insecure seals and were discarded. Preparations with air bubbles trapped within the drop were not used.

*From the University of Tennessee Medical School, Pathological Institute, Memphis, Tenn.

No attempt was made to record the percentage of sickling after hemolysis became evident or in slides in which the cells were fragmented. The percentage of sickled cells was determined for each reading by counting one hundred cells, using the high power objective, and noting the number of cells in this group having definite, pointed projections. The personal equation in the interpretation of what constituted a sickle cell was the same throughout the study, for the readings were all made by the writer, with the exception of an occasional count done under direct supervision. Where sickling was not uniform throughout the preparation, the percentage was determined on the basis of an average between the most sickled part of the field and the least sickled. In order to compensate somewhat for the variations in preparations from the same patient made under supposedly identical conditions, most of the readings were made on three or more moist preparations, and the average of these curves taken as representative of the individual.

In the selection of cases to represent the types designated as sickle cell

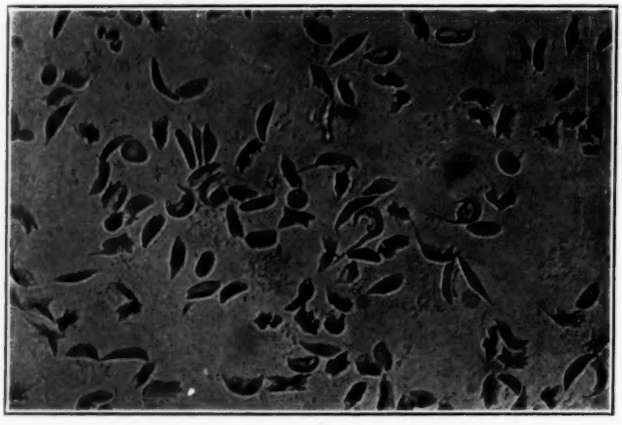

Fig. 1.—Microphotograph of a moist preparation from a case of sickle cell anemia, showing the characteristic bizarre and pointed forms.

anemia and sicklemia, the following criteria were used. The sickle cell anemia patients were all negroes having a marked anemia, characterized by definite blood destruction manifestations (jaundice, increased icteric index, urobilinuria) and an associated regenerative picture (immature erythrocytes, increased reticulocyte counts, leucocytosis, thrombocytosis, etc.). They all gave a history of previous attacks of ill health, of joint and abdominal pains, and of leg ulcers. At the time the studies were made, they were patients in the hospital with the classical anemia and febrile symptoms associated with the active phase of the disease.

The sicklemia patients were taken at random from a series of cases found to have the sickle cell trait when a survey was done to detect this anomaly. (This survey now includes the examination of 827 negroes, with an incidence of 8.2 per cent.) These patients had no history, signs, or blood picture suggestive of a hemolytic anemia. Although complete blood studies were not done on the cases in this group, stained smears and reticulocyte counts revealed no evidence of abnormal erythropoiesis.

One patient, a borderline case, who on admission showed the blood and clinical picture of sickle cell anemia, but who later presented no evidence of an active hemolytic process does not fit either group, and the curves for this case are given separately.

OBSERVATIONS AND DISCUSSION

The rate of sickling in 13 cases of sicklemia is given in Fig. 2. In two of these patients, the curve is taken from only one preparation, but in the others, three or more preparations are used in arriving at the composite curve for each individual. In Fig. 3 the rate of sickling in five cases of sickle cell anemia is represented. In each series the total number of preparations examined was 39. The average rates of sickling from these two groups are compared in Fig. 4.

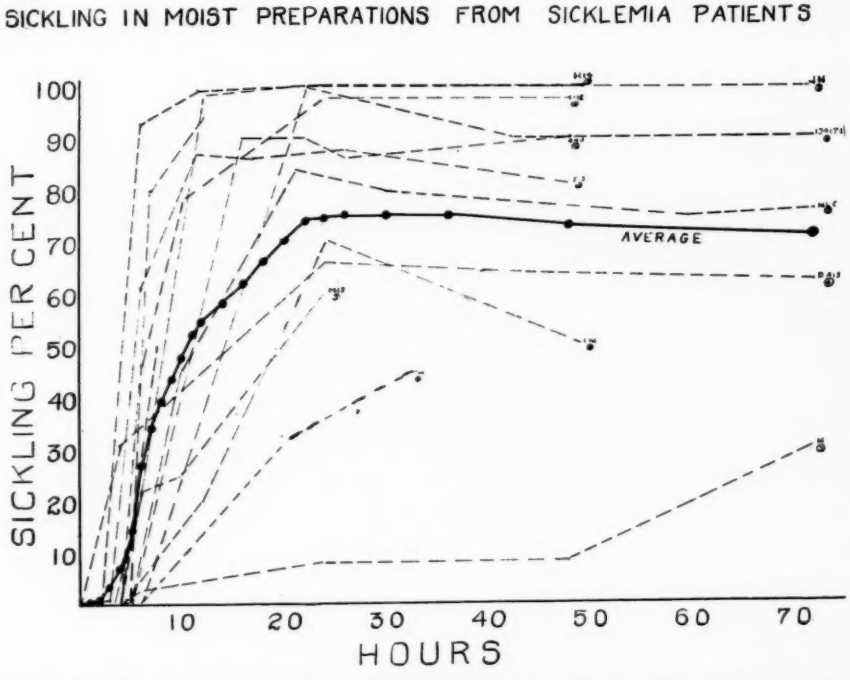

Fig. 2.—The rate of sickling in moist preparations from 13 cases of sicklemia.

From these observations, it is evident that in our series, sickled erythrocytes are often observed in moist preparations from sickle cell anemia patients as soon as the preparations are made, but are never present immediately in sicklemia patients. Moreover, the rise in sickling occurs sooner, and the total number of erythrocytes sickled at any given period during the first three days is greater. Although the return to the spherical form was not followed over a long enough period to compare adequately the speed of the rounding-up process in the two series, the tendency is for the percentage of sickled cells in both groups to decrease after twenty-four to thirty hours, with a sharper fall in the sickle cell anemia group.

These findings are in agreement with those reported by Huck,[3] who noted in patients with severe, mild, and with no symptoms, that the sickling percentage

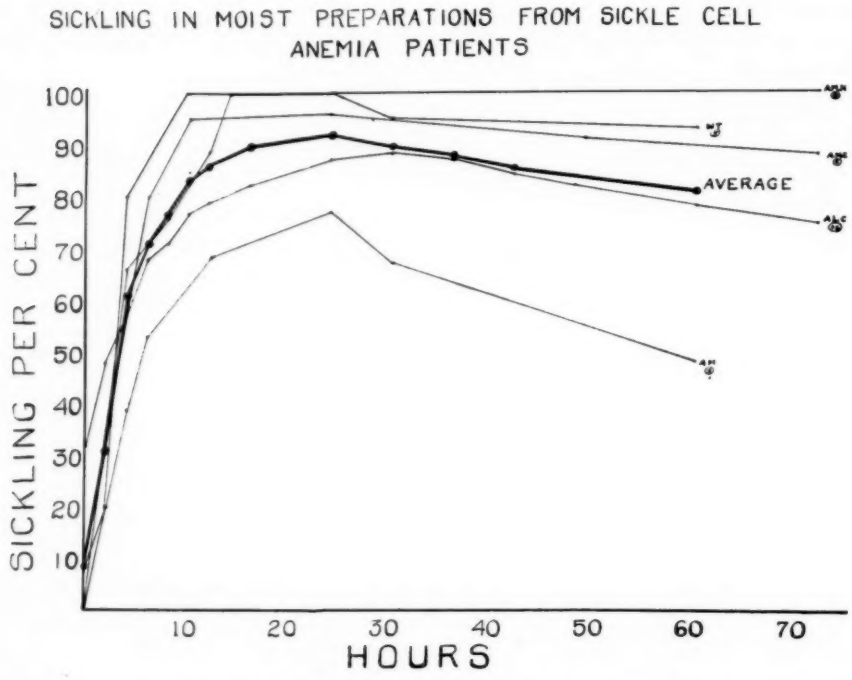

Fig. 3.—The rate of sickling in moist preparations from 5 cases of sickle cell anemia.

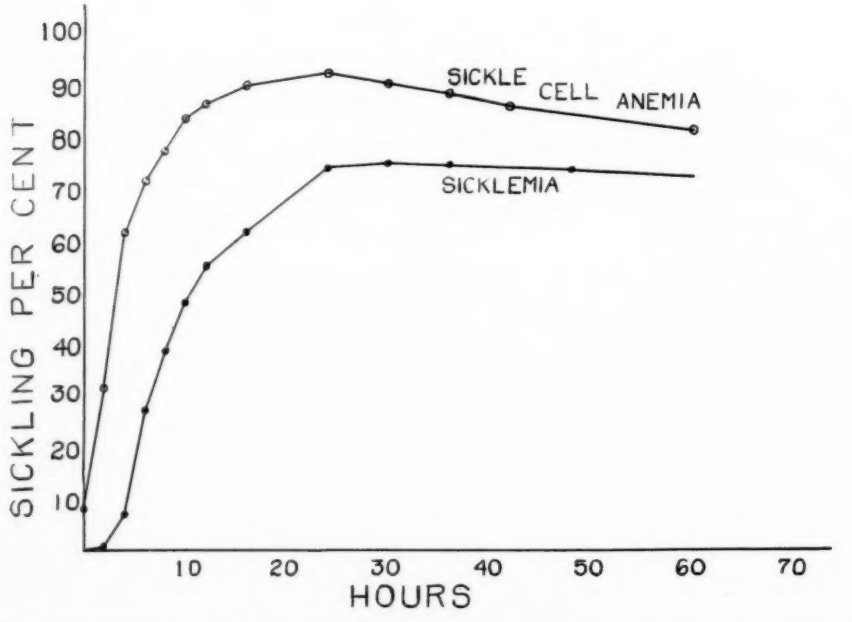

Fig. 4.—A comparison of the average rates of sickling in moist preparations in sicklemia and in sickle cell anemia cases.

was almost 100 per cent, 75 per cent, and 25 per cent respectively. Other workers have noted the same differences in anemic and nonanemic cases.[2, 7] The commonly observed fact that sickled cells are often found in smears from sickle cell anemia patients, and are not present in sicklemia patients is also in agreement with these findings.

Other observers, however, have failed to correlate the speed of sickling with the clinical state. Cooley and Lee[8] did not find that sickled cells occur more readily or in greater proportion in the blood of a sickle cell anemic child with a mild grade of hemolytic jaundice than in other children without symptoms. Stewart[9] stated that there is no "relation between the severity of the clinical picture and the percentage of sickled cells present." Hahn[10] noted that although

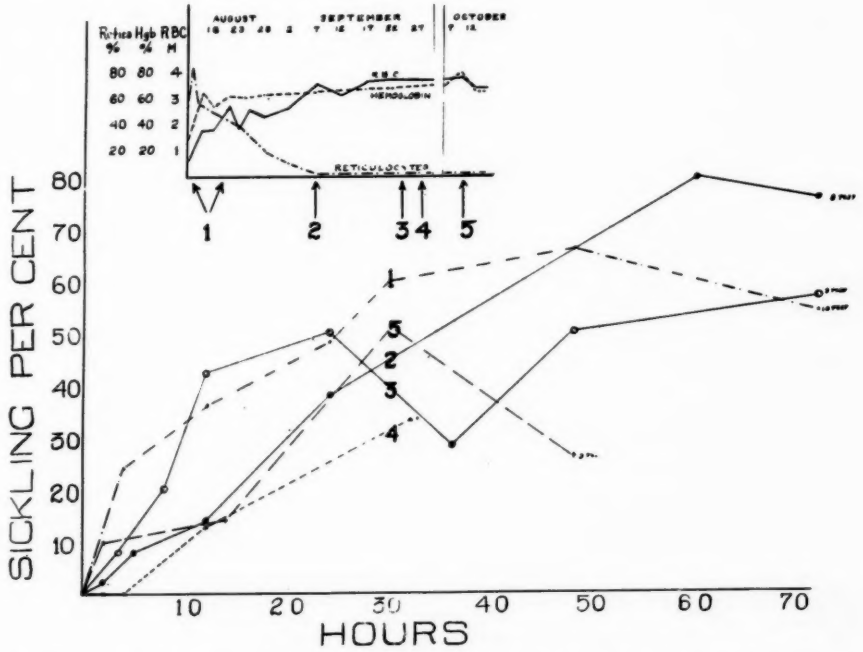

Fig. 5.—The rate of sickling in moist preparations from a mild case of sickle cell anemia, made at various times during a period of improvement.

sickle cell anemia patients have sickled cells in their circulating blood, "there is no difference in the ability of the red corpuscles of anemic and nonanemic subjects to form sickle cells outside of the body under suitable conditions."

In the interpretation of these seemingly contrary observations certain factors must be taken into consideration. The frequent finding of 100 per cent sickled cells after a sufficient interval of time in individual preparations from sicklemia patients as well as from cases of sickle cell anemia indicates that the capacity of the cells to sickle in the two clinical types under *suitable conditions* may be the same. In the moist preparation, however, it is not a question of how many cells could sickle under ideal sickling conditions, but what percentage of the cells assume bizarre forms under the conditions of the moist preparation. Also the variability of individual preparations and conclusions drawn from a few

observations may lead to false ideas as to the average reaction. Another, and probably a very important factor is that patients with sickle cell anemia vary considerably in their speed of sickling, and that mild cases or those with a temporary anemic episode of a hemolytic type may give the sickling charactertistics of the inactive group. Conclusions drawn from such a case would not hold for the more chronic cases.

The rate of sickling in such a mild case of sickle cell anemia is represented in Fig. 5. The patient, a negro girl of three was admitted with an erythroblastic type of anemia, with numerous nucleated red blood cells, a high reticulocyte and leucocyte count, and with an increased icteric index, etc. There were no typical sickled cells in the fixed smear, and the sickled cells in the moist preparations appeared slowly and in low percentages. The patient improved clinically, her hemoglobin and red blood cell count rapidly increased, the reticulocyte count dropped, and the immature erythrocytes disappeared from the blood picture. Series of moist preparations made during this return to the sicklemia type of condition showed no significant changes in the sickling rate. Although there persisted a low grade of anemia of the defective formation type, the patient after returning home showed no more clinical or actual evidence of anemia than a brother and a sister who likewise possessed the sickle cell trait, or another brother whose cells did not sickle in moist preparations. (The studies on this case during the active phase, were made during the extremely hot summer months when vaseline was a liquid and paraffin was necessary as a seal. This may have influenced the results, but we have noted no difference in the use of these two kinds of seals in other cases.)

Although the evidence is as yet limited, we are definitely convinced from our experience with a fairly large group of sickle cell anemia cases that the rate of sickling is related, not necessarily to the immediate severity of the anemia, but to the chronicity of the anemia. Those with no sickled cells in their circulating blood, with a slow rise in percentage and with a low maximum percentage have a better prognosis than the "high-immediate, rapid-rise, high-percentage" group.

The variability of moist preparations and the unreliability of a single moist preparation as representative of the average sickling rate for a given blood, as emphasized by Graham[4] and noted by other investigators, has been amply verified in our studies. Preparations quite often show variations in percentage in different parts of the same microscopic field. Sickling less than 1 per cent may be seen in one preparation while another made from the same patient at the same time under similar conditions may show 100 per cent. Sicklemia patients are more likely to show a more marked variability than are sickle cell anemia cases. In 194 preparations from cases known to possess the sickle cell trait, in which the rate of sickling was followed at frequent intervals for three days, 5 showed no cell changes. Although this is an error of less than 3 per cent, the fact that such a thing is possible is worthy of note. Preparations have been observed which showed no sickling in twenty-four hours, but became positive in fairly high percentages in forty-eight or seventy-two hours or longer. The difficulties in distinguishing sickled cells from other morphologic changes in erythrocytes, such as marked crenation, drying, and fragmentation phenomena poikilocytosis, elliptical cells and changes produced by pressure, may, at times.

be great. The present methods do not take into consideration such factors as method of collection, exposure to air, size of drop, character of spread, weight of the cover glass, hydrogen ion concentration of the blood after placement in the sealed preparation, bacterial growth, minor variations in temperature, exposure to light, and effectiveness of the seal. The fact that we cannot always produce the same curve in the same person is evidence of the presence of as yet uncontrolled factors, and clearly indicates that we will have to consider these sources of error in the interpretation of results and in the incidence of sickling as at present reported.

The use of the gas chamber method, as advocated by Hahn and Gillespie[11] as a means of experimental study has in their hands led to the most important fundamental observations concerning the sickle cell phenomenon, but in our hands it has been cumbersome and unreliable. Weeks spent in attempting to develop the technic to a point where it would be usable as a practical measure for conducting a survey, met with failure, although the feeble responses we obtained were in agreement with the findings reported by these workers. Graham,[4] Cooley,[12] and Scriver[12] have experienced the same difficulty. Others have reported positive results with the gas chamber method, but have not repeated the more detailed observations.

At the present time, therefore, we lack reliable methods. New procedures or a better control of the variable factors in the present methods are necessary before the interesting sickle cell anemia problem can be unravelled.

CONCLUSIONS

1. The erythrocytes in whole blood in moist preparations from patients with sickle cell anemia sickle faster and in a higher percentage than in sicklemia patients.

2. Twenty-four hours is the optimum time for the reading of moist preparations but negative readings at twenty-four hours do not eliminate the possibility of the sickle cell trait.

3. The rate of sickling is extremely variable, and conclusions that will be representative of the group or of the individual cannot be drawn from single cases or from a few preparations.

Appreciation is expressed for the skilled technical assistance given in this work by Miss Juanita Bibb, and for the cooperation of the clinical staff of the Memphis General Hospital.

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CLINICAL APPLICATIONS OF SUPRAVITAL STAINING*

EDNA H. TOMPKINS, M.D., NASHVILLE, TENN.

A COMPREHENSIVE definition of supravital staining is difficult. It implies, in general, the staining of living tissues independent of the organism as a whole. Specifically, it is used to indicate the staining of living cells which have been removed from the organism, or which are stained after the somatic death of the animal.

The physical and chemical laws which govern the suitability of substances for supravital staining are understood only in part. Cappell[3] gives an excellent definition of the practical working basis upon which this may be determined. He states that "the latter," i. e., supravital methods of study, "require agents which, while not toxic to the cells, are yet capable of producing their maximum effect within a few minutes," etc. The fundamental investigations of Schulemann[26] concerning the chemical and physical characteristics of vital dyes established the fact that a substance must belong in the borderland between colloids and crystalloids in order to be an efficient vital stain. This fact holds relatively true for supravital dyes. General laws concerning any other factors which regulate the suitability of a substance for supravital staining have not been determined. So far, the best supravital stains have proved to be weak basic dyes.

Simpson[27] made an extensive study of the suitability of a large number of both acid and basic dyes as supravital stains for blood. She took into consideration the toxicity of the dyes, the length of time necessary for satisfactory staining, the permanency of the color once staining had been obtained, the specificity of the staining for specific granules and for mitochondria, and the suitability of the dyes for revealing the vacuolar apparatus. She found that neutral red fulfilled the requirements of a satisfactory supravital dye for specific granules and phagocytic vacuoles better than any of the other dyes tested, and that janus green B was one of the best stains for mitochondria. Furthermore, she found that these two dyes in combination were especially satisfactory for depicting contrasts between mitochondria and vacuoles in blood cells. Sabin[24] also found these two dyes wholly satisfactory for the supravital staining of blood, and more satisfactory than the other dyes which she used. Utilization of these two dyes by other investigators in the field of supravital staining has yielded equally satisfactory results, so that, at the present time, these two stains, either alone or in combination, have come to be the chief dyes commonly used in the supravital study of the blood. They are the only dyes which will be considered in the discussion which follows.

The technic of preparing the slides and solutions and of making the supravital smears has been given in detail elsewhere[8, 9] and need not be repeated here. In brief, the method consists of flooding clean glass slides with dilute alcoholic

*From the Department of Anatomy, Vanderbilt University School of Medicine.

solutions of either neutral red alone, or of a combination of neutral red and janus green. The slides are at once drained and dried, and are then ready for use. A drop of fresh blood is collected on a coverslip, which is immediately placed on one of these stained slides. The blood must be allowed to spread by capillary attraction and without the aid of pressure. The edges of the preparation are rimmed with vaseline to prevent evaporation, and the smear is kept in a hot box at body temperature long enough to permit staining. Five or ten minutes are sufficient for the staining of the mitochondria. About twenty minutes are required for satisfactory staining of the vacuoles and granules. Janus green is quite toxic, and it is therefore necessary that blood stained with it be studied soon after preparation. It also inhibits, to some extent, the staining with neutral red, so that an accurate determination of the size and number of the vacuoles must be made with neutral red alone. Furthermore, any estimation of the vacuolar activity must take into consideration the length of time that the blood has stood in contact with the stain, as the vacuoles increase in number, size and P_H reaction with time. We have published elsewhere[8, 9] the exact requirements for a satisfactory supravital preparation as well as descriptions of the appearance of the normal white blood cells when freshly stained, and after having stood for various periods of time. These reports contain very accurate illustrations in color of the cells stained both with neutral red alone and with neutral red in combination with janus green. It seems wiser to refer any one wishing to study exact descriptions of supravitally stained cells to those articles than to attempt at this place a repetition which, to be of any value, would have to be lengthy.

It can be seen from the foregoing brief review of the requirements for satisfactory supravital staining that there are some inherent disadvantages in the method. At first thought, these may make the method seem impracticable for any very general use, especially as some of the disadvantages do not occur in other methods of studying blood.

The chief disadvantage in the method is, perhaps, the fact that the smears cannot be kept long and therefore must be studied soon after they are made and cannot be saved for future reference or for shipment. The necessity of studying them within a given period of time sets an inflexibility that often may be difficult to meet. This is especially true if the preparations must be transported to any distance. Furthermore, transportation under any condition is somewhat difficult, as the slides must be kept flat to prevent flowing of the cells to one side of the preparation. In fact, in most cases, the method does not lend itself to instances where the blood is to be examined at any great distance from the place where the preparations are made.

The need for a hot box or some other method of keeping the blood at body temperature may also at times cause embarrassment. Where the microscope is kept in any permanent place and the blood brought to it, this offers no problem, as a permanent heating system can easily be set up. It is only in instances where the microscope is carried to the blood that there may be difficulty. This difficulty can be met by the use of a portable hot stage.

Preparation and staining of the smears are without any doubt simpler and less subject to error than is the case with fixed smears; but the preliminary clean-

ing of the glassware is somewhat time-consuming. For properly made fixed smears, to be sure, the coverslips need as careful cleaning as for supravital preparations. But in the latter case, the slides must also be subjected to the same careful attention. Since it is as easy to clean and stain a larger number of slides as a few, the time required for their preparation can be materially minimized by the preparation of a large supply. They can be stored indefinitely if kept away from dust and grease.

More time and skill are necessary in learning to use the supravital method to its full advantage than is the case with methods of fixed staining. This is simply because the method depicts more cytologic factors and permits of the detection of more modifications in those factors than do other methods. For simple differential counting the method may be learned as quickly as any other, but for detection of all the possible pathologic modifications that may be observed in the various factors that stain supravitally and for their interpretation, considerable time and experience are necessary. It is much like the fact that it does not take long to learn the name and appearance of an intricate machine, but it requires considerable experience to learn all of the uses to which it may be put, and all of the factors that may go awry with it. The supravital method of staining blood offers a tremendous range of factors for study.

There is one fact, however, which may offer difficulty for even routine differential counting. This is the fact that nuclei do not stain and therefore do not aid in the rapid detection of the nucleated cells within the field of vision. This is of no significance in the detection of the granulocytes and monocytes, since their granules and vacuoles, respectively, make them conspicuous. It does, however, make detection of lymphocytes and nucleated red cells more difficult than with fixed staining, where the nuclei are prominent. In the case of the red cells this is true with both the single and double staining. In the case of the lymphocytes, it is true only with the single stain, since mitochondria aid in the detection of lymphocytes with the double staining. To a trained observer the lack of nuclear staining is not a source of error, as both lymphocytes and nucleated red cells are easily visible in a supravital field when the source of illumination is properly adjusted and the smears are thin enough. Moreover, in most instances, especially if the preparation has stood twenty or more minutes, the lymphocytes contain a few tiny vacuoles of neutral red which serve to bring them to the observer's attention.

Another objection to the use of the supravital technic in the study of blood is the fact that basophilia and stippling cannot be detected as such. Key[15] has shown that the material, which can be demonstrated in young red cells by the appropriate use of certain supravital stains, is the same substance which appears as stippling and basophilia in fixed smears. Reticulation may appear in some of the red cells in a supravital preparation after they have stood in contact with the stain for a considerable period of time; but for any accurate estimation of reticulation a concentration of neutral red must be employed which is too great to be compatible with the life of the cell, and therefore with supravital staining.

Parasitic inclusions (in particular, malarial parasites) also do not stain in supravital preparations until after a long exposure to the dye, and therefore

may easily be overlooked at the time of the differential count. When they do finally take the dye, they are very prominent.

It will be seen from the foregoing list of the disadvantages of the supravital technic that the principal ones are the impermanency of the preparations, the necessity of examining them soon after they are made, the impracticability of transporting them, and the difficulty of detecting parasitic inclusions and certain pathologic features in circulating red cells, i. e., nuclei, stippling, basophilia.

In a consideration of the use of any particular technic, it seems reasonable to consider its advantages and the information obtained from its use, as well as its disadvantages, and to contrast these with the advantages, disadvantages and information inherent in other related technics. In the case of the supravital technic for the study of blood, a number of cytologic characteristics can be demonstrated which cannot be seen in fixed preparations. Furthermore, some of the characteristics which can be seen in fixed preparations appear in a somewhat different manner in fresh preparations. It is necessary to know wherein the cytologic features presented by the two methods vary, and in what clinical conditions any particular set of features is of importance before one can draw any conclusions as to the technic of choice for the study of blood in general, or in any particular instance.

In supravital preparations the cells are living and may be considered more or less in the physiologic state in which they occurred in the body at the time that they were withdrawn. This statement, of course, holds true the sooner the preparation is examined after it has been made. For that reason it is considered important to study the cells as soon as they have taken sufficient stain to bring out their cytologic features. As has been mentioned elsewhere, this is from twenty to thirty minutes after preparation of the smear. It is obvious that a study of any physiologic characteristics that can be made observable might be of diagnostic and prognostic aid wherever pathologic states of the host cause modifications in those characteristics, or where the cells are inherently pathologic in themselves.

The important physiologic factors which are demonstrated by the supravital technic, and are not demonstrated in fixed preparations, consist of motility, mitochondria and the vacuolar apparatus. The motility varies in rate and character in the different types of cells, and therefore can be used to help in their differentiation. The rate of motility in all of the cells is subject to considerable variation due to trauma in making the smears, to the temperature at which the smears are kept, to the age of the cell, to pathologic conditions of the cell, and finally to factors in the environment which are not understood. Obviously, where any activity may be affected by so many factors, and where those factors are only partly controllable, decisions based upon that activity can be made only with reservation. In the case of cellular motility, therefore, the increased or decreased activity, of any strain of cells, can be judged only in relation to the activity of other cellular types in the same smear. So far as has been reported, there is no evidence that the form of motion in any given type of cell changes. It is merely the rate of activity which seems to be modifiable. In the differentiation of cellular types, the absence of motility is of very little signifi-

cance, as cells may remain quiescent for long periods. The presence of motion, on the other hand, may be of considerable assistance. This is especially true in the case of the mononuclear cells. Cunningham, Sabin and Doan[5] found that both monocytes and lymphocytes were motile, while, on the other hand, they never observed movement of myeloblasts or of myelocytes until the cells had practically become adult polymorphonuclear cells.

The staining of the vacuolar apparatus in blood cells is perhaps the most valuable feature demonstrated by the supravital technic. The aid offered by the vacuolar apparatus in the differentiation of mononuclear white blood cells has received considerable attention; but the importance of the modifications within the vacuolar apparatus under various pathologic conditions is just beginning to receive recognition and to be considered of diagnostic or prognostic help. The staining of the vacuoles in monocytes is particularly prominent, and particularly subject to modifications. Simpson and Sabin showed that the vacuolar apparatus in the monocytes is very distinctive and serves as an accurate differentiation of monocytes from lymphocytes or from young mononuclear cells of other series. They stressed the number of vacuoles and the ground glass appearance of the cytoplasm in the monocyte as offering a marked contrast to the few vacuoles and the clear hyaline-like cytoplasm of the lymphocytes. To these major features in the characterization of the monocyte, Sabin added the size and arrangement of the mitochondria, the quality of the unstained nuclei, and the tendency to rosette arrangement of the vacuoles. Cunningham, Sabin and Doan, and Cunningham and Tompkins[8, 9] agreed in the main with their descriptions, but showed that there is considerable pliability in the vacuolar arrangement dependent on whether the cell is rounded up or in motion. All of these authors found a much more extensive vacuolar apparatus in the monocytes (i. e., both more and larger vacuoles) than in the lymphocytes. While there has been no disagreement with the descriptions of monocytes given by the above authors, various reports show that the individual characteristics of the monocyte are not specific to that cell alone, but may be observed in other mononuclear cells of the blood when supravitally stained and cause confusion in their differentiation. The possibility of confusion between monocytes and lymphocytes has been particularly stressed. Bloom[2] studied the mononuclear cells in the blood and tissues of rabbits following experimental irritations. He found many cells which exhibited various stages of vacuolar activity and in which differentiation between lymphocytes and monocytes was impossible. Lawrence and Todd[18] studied the blood in various clinical conditions in which the lymphocytes were admittedly involved, and found many lymphocytes which had such an overactive vacuolar apparatus that they could not be differentiated from monocytes on the basis of the vacuolar apparatus alone. Hall[14] published a very careful and critical review of the hematologic literature concerning the results of supravital staining. In particular, he examined the evidence dealing with the value of the supravital technic in the differentiation of monocytes. He concludes that the method does not help in the differentiation. Among other factors which led him to this conclusion was the extreme modifiability of the vacuoles and the ease with which their character is changed by pathologic and

environmental conditions. As reports of supravital studies of pathologic cases appear, the extent to which the vacuolar apparatus may be modified becomes more evident. At the same time, the probability arises that this liability of the apparatus may be of great value in differential diagnosis. Sabin showed that the vacuoles of the monocytes in Malta fever varied considerably. Wilson and Cunningham,[30] Rucks and Cunningham[23] and Lawrence, Josey, and Young[17] found all stages of development of the vacuolar apparatus in cases of monocytic leucemia. Cunningham and Tompkins,[7] Blackfan and Diamond[1] and Rogers[22] reported specific changes in the vacuolar apparatus of the monocytes in tuberculosis. Comparable variations have been observed in the vacuolar apparatus of lymphocytes under various pathologic conditions.[30, 18] Tompkins and Cunningham[29] have reported a variety of conditions in which they found modifications in the vacuolar apparatus of either monocytes, lymphocytes or granulocytes. In all of these reports, the modifications of the apparatus were regarded as specific to the pathologic conditions in which they occurred, and while they rarely offered any confusion in the differentiation of the cells, they were found to be of assistance in diagnosis.

The third factor of major importance demonstrated by the supravital technic in the study of blood cells is the staining of the mitochondria. Mitochondria, of course, have been demonstrated by methods of fixed staining, but these have not permitted of the simultaneous study of a sufficient number of other cytologic features. The simultaneous staining of the mitochondria and vacuoles in supravital preparations has a value that studies of the mitochondria alone in fixed preparations do not offer. It is obvious, in the first place, that simultaneous observation of a number of factors within a cell is preferable to the necessity of separate observations in different cells of the same type. Furthermore, it is obvious that there is less distortion of the intracellular inclusions in stained living cells than is likely after even the most felicitous fixation. As the size, shape, and arrangement of the mitochondria have been considered as important as their number in cellular differentiation, this feature assumes considerable value. Even under the conditions of supravital study of the mitochondria by the use of janus green, there has been found to be considerable variation in their number and form from cell to cell of a given type. Cowdry[4] observed that mitochondria are extremely sensitive to physiologic and pathologic conditions. Despite their normal variations, however, there has been found sufficient similarity in the mitochondria in any one type of cell and sufficient difference in the mitochondria between types for a differentiation of blood cells to be made which is based partly on the mitochondrial pattern. This is especially true when neutral red is used in conjunction with janus green. By the use of these two dyes, together, Simpson and Deming[28] and Sabin, Austrian, Cunningham and Doan[25] were able to trace the development of granulocytes from myeloblasts through successive stages to the mature polymorphonuclear cells, and to define the myeloblasts and young myelocytes with a certainty not previously obtained. With the same technic, Cunningham, Sabin and Doan followed the development of leucocytes, lymphocytes, and monocytes from their stem cell. They discovered very useful and consistent differences in the cytologic

patterns of the young cells of each series which have made it possible to distinguish them from each other, and from mature myelocytes, lymphocytes and monocytes. As in the case of the vacuolar apparatus, the variations which may occur in mitochondria, due to both physiologic and pathologic causes, may be of considerable diagnostic and prognostic assistance rather than a source of confusion. Cowdry realized this and discussed the probable importance of the sensitivity of mitochondria to pathologic changes. Taken alone, without consideration of the other cytologic factors stained by neutral red, the character of the mitochondria in the different cells and their modifications might well cause confusion. Studied in combination with other factors, the mitochondria are invaluable. In fact there are a considerable number of conditions in which the mitochondria have been found to be specifically characteristic.[30, 18, 29]

From the foregoing remarks it is obvious that supravital staining permits of observations of motility, vacuolar apparatus and mitochondria, and that these three factors are subject to modifications under various environmental, physiologic, and pathologic states. Despite the modifications, the pattern of these three factors in each type of cell seems sufficiently characteristic to be of distinct value in the differentiation of these cells. The aid obtained in cellular differentiation is of especial value in the case of the young mononuclear cells. On the other hand, the modifications in the mitochondria and vacuolar apparatus are sufficiently specific to the conditions which elicit them for them to be of aid in the differential diagnosis.

In addition to these three major factors brought out by the supravital technic, there are various other factors which may be observed with other technics, but which at times may be more accurately measured in the living cells. These include measurements of the diameter of cells, abnormalities in the shape of cells, the color of red cells, and the presence of any cytoplasmic inclusions, such as fat, which might be destroyed with fixation. The distortions in the shape of cells which often occur in pulled smears are avoided in living preparations, where the cells are free to assume their natural shapes. This feature has its greatest importance in the detection of sickle shaped red cells, which Lawrence[16] found to be more reliably distinguished in fresh than in fixed preparations. The production of "smudges" which often results from the trauma of pulling smears, especially in pathologic bloods with very fragile cells, is also avoided in supravital preparations. It is obvious that the elimination of smudges adds to the accuracy of the differential counts, and of the detection of pathologic cells.

Estimations of the color of red cells can be made with extreme accuracy in supravital preparations in which basophilia and variations in the intensity of stain do not interfere with the color due to the hemoglobin alone. In addition, it is easy to detect the faint traces of hemoglobin which occur in the very young red cells, and which are concealed by intense basophilic staining. The detection of hemoglobin simplifies the differentiation of very young red cells from young white cells.

Tompkins and Cunningham found that fat occurs occasionally in the white blood cells under certain pathologic conditions and may aid in diagnosis or prognosis. They found that in extremely toxic infections, and especially in

moribund states, the polymorphonuclear neutrophiles may contain highly refractive, unstained droplets which they consider to be fat. In two cases of generalized lymphosarcomatosis they found similar droplets in the lymphocytes. Cunningham, Sabin, Sugiyama and Kindwall[6] found that fat was consistently present in degenerating epithelioid cells.

From this review of the important features connected with the supravital technic, one is in a position to analyze the conditions in which the specific information obtained from supravital staining is likely to be of help in diagnosis or prognosis. It has been shown that the method is of value first in the differentiation of mononuclear cells of various types, including the young forms of the different strains of white blood cells; and secondly, in showing qualitative differences in any single type of mononuclear cell. In this latter respect, the method is particularly helpful in depicting qualitative changes in the lymphocytes and monocytes. Therefore, the method is likely to give assistance in any condition involving the mononuclear cells. This is true, whether it is a condition involving the lymphocytes or monocytes, or a condition associated with the appearance of young cells.

In the case of diseases involving the lymphocytes, it has been found that the increases in those cells in certain diseases, such as pertussis,[18] lymphocytic reactions to sepsis,[30, 18] and tuberculosis,[7] are due to cells that are normal in every respect, while other diseases are characterized by the appearance of lymphocytes which stain in the manner of young cells. The infections of childhood, typhoid fever, malaria, and measles[29] are the common conditions in which young lymphocytes may be found. On the other hand, the lymphocytes in infectious mononucleosis are different from either normal or young cells.[30, 18, 29] The lymphocytes of lymphatic leucemia have been shown to exhibit characteristic changes in mitochondria, vacuolar apparatus and nuclei which help to differentiate them from other conditions involving the lymphocytes, as well as from other mononuclear cells.[30, 18, 29]

Supravital staining has proved especially valuable in diseases involving the monocytes, because it has aided not only in the detection and differentiation of these cells, but has revealed sufficiently characteristic changes in them in a number of pathologic conditions for the modifications to be of value in diagnosis. Morriss and Tan,[20] Cunningham and Tompkins,[7, 29] Blackfan and Diamond,[1] Rogers[22] and Finner[11] have found the monocytic count and the relationship of monocytes to lymphocytes to be specifically involved in tuberculosis. Sabin found the monocytes numerically and qualitatively changed in Malta fever. Mulligan[21] and Tompkins and Cunningham found them affected in malaria. The latter authors found them stimulated in their capacity to store dye in a group of conditions with associated hepatic disturbances, and also as an accompaniment of pyogenic infections. They also found them depressed in their capacity to store dye in conditions associated with anemia. The diagnosis of a third type of leucemia, i. e., monocytic leucemia, has been made more certain by supravital blood studies.[30, 17, 23] As a result of these studies it has been possible to accurately differentiate the condition from lymphatic and myelogenous leucemia.

In fact, in the leucemias in general, supravital staining with the double stains has proved of tremendous value in the separation of the three main types, and in the classification of those types according to their chronicity. Detailed descriptions have been published of myeloblasts and myelocytes at various stages of maturity in the blood of cases of myelogenous leucemia, and the points have been discussed by which they may be differentiated from other mononuclear cells.[28, 25, 29] It has been found that myeloblasts and very young myelocytes characterize the more acute stages of the disease, while late myelocytes are characteristic of the more chronic stages.[25, 29] It has also been found that an accurate estimation of the number of young myelocytes, compared to the number of late myelocytes, is of help in distinguishing severe reactions to pyogenic infections from aleucemic stages of myelogenous leucemia.[29]

It is impossible to lay down dogmatic rules as to when any one method of studying blood should be employed. There are times, as every one knows, when a number of methods are necessary to give enough information for diagnosis. There are times when any satisfactory method will give all of the necessary information. In regard to the use of the supravital technic, it would seem as if its value in the analysis of mononuclear cells should, wherever possible, make the technic a routine in all diseases involving the lymphocytes and lymphoid tissues, in all questions of leucemia of any sort, and in all conditions in which the number or character of the monocytes have been shown to be of diagnostic aid. These latter conditions include tuberculosis, malaria, anemias and conditions accompanied by hepatic disturbances, such as arsphenamine toxemia, catarrhal jaundice, and Malta fever.

Besides these special cases, when supravital studies seem routinely indicated, the method should also be employed, in addition to fixed staining, wherever fixed staining has given indications of abnormalities of the mononuclear cells, or of the presence of young white cells.

In the study of anemia, the method can also be profitably employed at times for accurate estimations of the color of the red cells, as well as for determinations of the size and shape of the cells, and for detection of the very young red cells, the megaloblasts. It is especially indicated where there is any question of the presence of sickle cells.

In the course of acute infections, the method is useful in differentiating the infectious conditions from aleucemic myelogenous or lymphatic leucemias. In addition, in infections, the method may give information concerning the toxicity of the infections, and concerning the activity and youth of the granulocytes, and therefore of the regenerative powers of the marrow.

With the exception of the above conditions, when supravital studies seem specifically indicated, there is no reason to prefer them. Fixed preparations are of course necessary when permanent smears are desired.

In addition to the use of the supravital technic in the study of blood, the method offers some distinct advantages in the study both of body fluids, and of scrapings of various tissues. The possible applications of the method in these directions have but just begun to be investigated and very little has as yet been published concerning such studies. Cunningham and Tompkins[7] found that

supravital studies of tuberculous exudates showed great numbers of epithelioid cells and were of diagnostic importance. McJunkin[19] made supravital studies of the lymphoid tissues in Hodgkin's disease. Forkner[12, 13] made an extensive investigation of both normal lymph nodes and nodes from various pathologic conditions, including lymphosarcoma, Hodgkin's disease, lymphatic leucemia, and tuberculosis. He devised a very simple method for biopsy extraction of enough material from the nodes for supravital studies, and found sufficiently characteristic changes in the tissues under the different pathologic conditions to encourage further investigation and a restricted application of his methods to selected cases. Doan[10] applied the method to the study of marrow from cases of pernicious anemia and obtained invaluable information concerning the phagocytic cells and the regeneration of the red cells.

The technic for such studies is essentially the same as that for blood. The strengths of the dyes which give the most satisfactory results vary according to the tissue to be studied. Injury to the cells must be avoided in making the preparations. Smears of fluids are made in the same manner as those of blood. Smears of tissue scrapings are also made in the same manner whenever they are moist enough in themselves to permit of spreading. Pieces of solid tissue must be carefully avoided and pressure is fatal to the cells to be studied. Where the tissues are not sufficiently moist to spread normally, the scrapings may be mixed with a drop of homologous serum and smears made from the mixture. Saline solutions are not suitable for this purpose, as they cause too great an edema of the cells, with a consequent distortion of the cytologic figures.

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HEMOPHILIA*

C. A. Mills, M.D., Cincinnati, Ohio

HEMOPHILIA is a condition characterized by a delayed clotting time of the blood and a history of repeated hemorrhages, which is found only in males but is transmitted through the female as a sex-linked character. Thus, it will not appear in the children of a hemophilic man but may be transmitted by his daughters to appear in their sons. The disease will not appear in the sons of any male descendant of a hemophiliac (except in case of intermarriage between two such families) but may crop out at any time in the sons of the female descendants. This law of hereditary transmission of the disease was first promulgated by Nasse in 1820[1] and has since then received repeated verification, especially by Bulloch and Fildes[2] in their critical investigation of the literature of reported family histories. Gates[3] has very recently given an excellent survey of the literature along this line which should be read by anyone particularly interested.

Each hemophilic male, therefore, who marries and has children serves as a new starting point of the disease in a hereditary sense. This is well illustrated by a family history which I recently presented.[4] The drawing of this family tree is reproduced here to emphasize the law of transmission of the disease. Widespread diffusion of the disease in recessive form, which has taken place with the passage of centuries, no doubt accounts for the numerous cases seen in which no positive family history is obtainable. There is no way of being certain, however, that it does not occur spontaneously. Intermarriage between members of different or of the same hemophilic family usually proves disastrous, as the disease at once becomes rather dominant in character. This has happened in several of the royal families of Europe through their close intermarriage.

Etiology.—Our knowledge of the origin of the disease is no further advanced than it was a century ago. Pickering[5] found certain points of similarity between hemophilic blood and that of certain embryonic forms, and obtained beneficial results from liver feeding in two cases. He therefore postulated some kind of faulty liver development as a basis for the condition. Marlow[6] has recently repeated Pickering's attempt at liver therapy in 4 cases, but with no success whatever. It is therefore unlikely that the liver is concerned in the disease, although it is intimately related to blood clotting factors in other ways.

Perhaps the most important work so far reported on the etiology of hemophilia is that of Birch.[7] Working on the assumption that females of hemophilic families must possess some protective factor against the disease, which is lacking in the males, she tried ovarian transplants and ovarian extract therapy in hemophilic males. Apparently remarkable success has attended this type of therapy. She also went one step further, and tested the urine of hemophilic males for the

*From the Department of Internal Medicine, University of Cincinnati.

small trace of female sex hormone which is usually found in male urine. In her five hemophilic cases no trace of this hormone was found. She, therefore, believes that the disease is kept in recessive form in the female carriers by the activity of their sexual organs, and that it crops out in certain male members of the family because of an entire lack of the small amount of female sex hormone normally present in males. Much work remains to be done before her proof is complete, for instance whether there are nonhemophilic males who excrete no female sex hormone in the urine.

Pathology.—Most of our knowledge of the pathology of hemophilia is concerned with the coagulation of the blood. Attempts to show the presence of undue friability of the capillaries, or alterations in the coagulative property of the tissue juices, have yielded no convincing results.

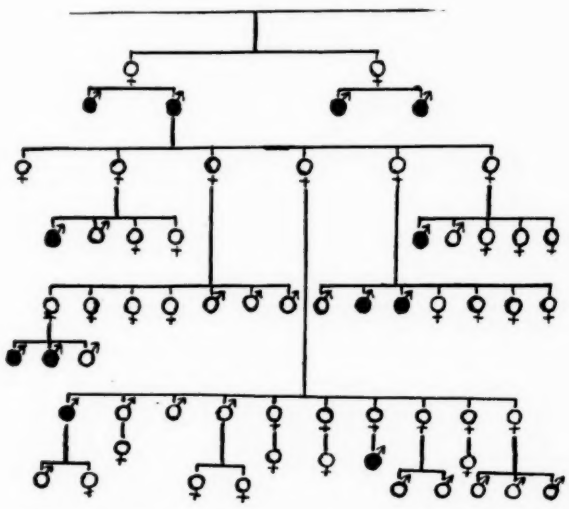

Fig. 1.—Genealogy of the Prickett family, with bleeders shown in black. No earlier history was obtained.

The *blood* exhibits as its major abnormality a *prolonged clotting time.* This may vary anywhere from normal limits up to several hours, depending on the severity of the condition and the time at which the blood is taken. One person may show wide fluctuations of his clotting time at different periods of the year, at one time being apparently normal and a few weeks later going into a severe hemorrhagic state with greatly delayed clotting. This marked variability in the clotting time is characteristic of the disease and should always be kept in mind. The *bleeding time* from a stab wound in the finger or ear is usually normal,[8, 9, 10] although at times it becomes greatly prolonged if the wound be treated a bit too roughly. The cellular elements of the blood are normal in character and amount except for the secondary anemia, following severe bleeding. Recovery from this anemia is usually very prompt. The blood *platelets* are normal in number and size but exhibit a delayed clumping and disintegration which has been considered by some workers[10, 11] as an important factor in the prolonged clotting time. There has been some disagreement[10, 12] as to whether the platelet

material liberated by the disintegration, possesses the coagulative power that it normally should.

Prothrombin of the blood in hemophilia seems to be the only clotting factor exhibiting definite abnormality. Howell[11] believes that there exists a reduction in the amount of prothrombin which accounts for the delay in thrombin formation and clotting. However, the experiments of Addis[12] seem more conclusive, showing only a delay in thrombin production, with no deficiency in the total amount finally formed. Mills[13] likewise concluded that the fault lay, not in the amount of prothrombin or thrombin, but in the delayed activation of the former to the latter. He found prothrombin in hemophilia to be much more resistant to activation with cephalin than in normal blood, and that this fault was corrected by the induction of a local skin protein reaction. *Thrombin* of hemophilic blood, when once formed, is normal in amount and action. It possesses the proper capacity for clotting either normal or hemophilic fibrinogen. *Blood fibrinogen and calcium* have been found normal in amount and character by all who have studied them in this condition. The presence of *anticoagulants* in the blood, to account for its delayed clotting, has received much study, but again there is almost unanimous agreement. There is no excess antithrombin present[10, 13, 14, 15, 16, 17] nor is antiprothrombin increased in amount.[18, 19]

Other pathologic findings in hemophilia usually include marked dental caries, with root abscesses, and chronic joint changes. These, however, more properly come under the heading of complications of the disease. The only other pathologic condition found is the entire lack of female sex hormone in the urine of these patients. The work of Birch[7] in this connection needs confirmation by other workers and on a larger series of patients.

Symptoms and Signs.—Repeated severe hemorrhages furnish the outstanding feature of the disease. These usually start in early childhood, rarely however before two years of age, and continue throughout childhood. Adolescence usually brings a measure of relief, and by early adult life the hemorrhages are in most cases fewer and less severe. The severity of the bleeding lies not in the rate of blood loss, but in its duration. Frequently the loss is only through seepage around clots, but the continuance of this for ten days to two weeks brings the patient down to a critical level of anemia. It would seem that the only spontaneous means of stopping the bleeding in many cases lies in closure of the wound by healing processes rather than through the clotting power of the blood. Bleeding most frequently occurs from the *gums,* with the shedding of the deciduous teeth or tooth extraction, from a *torn frenum* in the mouth, into the joints, especially the knees, elbows, and shoulders, and from *traumatic* injuries to any part of the body. Subcutaneous and intramuscular extravasations from injury are rather common. Probably the most difficult hemorrhages to control are those from the gums and frenum, while those in the joints cause most discomfort to the patient and lead to the greatest harm through the joint changes produced.

There are no characteristic physical findings in hemophilia except those resulting from the bleeding tendency. Because of the danger attendant on extraction of teeth, together with a rather poor state of nutrition in these persons,

dental caries is usually marked and root infections are common. Joint deformities with varying degrees of limitation of motion are common. Anemia is seen, of course, during and immediately after the hemorrhages, but recovery is nearly always prompt, once the bleeding has ceased.

The principal sign of the disease is the delayed clotting of the blood with the frequent and prolonged hemorrhages. The clotting time may range anywhere from normal limits up to several hours and varies at different periods as discussed in a previous paragraph. One expression of this delayed clotting is the *prolonged prothrombin time*, as described by Howell[13] and verified by numerous other investigators.[9, 14, 20, 21] The bleeding time is normal, as are also the cellular elements and platelets of the blood.

Course of the Disease.—Manifesting itself rarely before the second year of life, the bleeding tendency is most severe through early childhood, usually becoming less troublesome toward the beginning of adolescence. By early adult life it often becomes quite dormant or disappears almost entirely. In a few cases it persists in severe form throughout life. In no patient does the disease maintain the same severity at all times. All hemophilic patients have periods when their bleeding tendency is lessened or gone completely. But these periods may suddenly change over into ones of great severity. These periods of varying intensity usually cover weeks or months. Very often in the time of betterment the patient will seek dental or surgical aid without acquainting the dentist or surgeon of his disease, and find himself suddenly in the severe phase of hemophilia coincident with the operative procedure. Great caution must therefore be used in carrying out any operative procedures on patients with a positive personal history of hemophilia, no matter how little trouble they have had in recent months, nor how normal the blood coagulability has recently been.

Complications.—Dental caries and root infections, together with the accompanying gingivitis, form the most common complications of hemophilia. They should be classed as complications because they usually result from neglect of the teeth and gums through fear of hemorrhage. A mild degree of malnutrition is usually present, whether from the abnormal life led by the person, from oral infection, or from the repeated hemorrhages, is difficult to say. Chronic joint changes, resulting from repeated bleeding into the joints and the presence of a low grade infection, usually end in hypertrophic arthritis and varying degrees of limitation of motion.

Diagnosis.—Diagnosis of hemophilia rests mainly on three points: (a) positive family history as given earlier in this article, (b) personal history of repeated hemorrhages beginning in early childhood, and (c) a prolonged clotting time of the blood. In many cases the family history is negative or lacking and diagnosis is made on the other two points. The personal history of repeated hemorrhages is so important in the diagnosis with patients past early childhood that one should hesitate to class as hemophilic a patient with an initial severe hemorrhage without going into a careful study of the blood coagulability. When presented with an initial hemorrhage in early childhood, however, diagnosis must often rest entirely on studies of the blood coagulability. In such a case the determination of the clotting time is important. Many methods are available

for this purpose, the simplest of which are probably the test tube and the capillary tube methods. A description of the technic of the latter method, together with its factors of error and the normal fluctuation of the clotting time will be found in an article by Mills and Peterson.[22] A marked delay in the clotting time of the blood, but with the eventual formation of a clot which contracts normally, and the presence of normal bleeding time and normal number of blood platelets would favor the diagnosis of hemophilia.

Differentiation from other hemorrhagic conditions is of course necessary. The question is most often raised in cases of purpuric hemorrhages, especially the repeated, severe, and protracted hemorrhages of essential thrombocytopenic purpura. Differentiation here depends on the presence in purpura of a normal or slightly prolonged clotting time, an abnormally long bleeding time, a marked reduction in the number of blood platelets and the production of a nonretractile clot. Accurate diagnosis is especially important in those cases of essential purpura in which splenectomy is being considered, since a mistake might well mean a fatal outcome. Acute leucemia in childhood is sometimes accompanied by hemorrhages which require differentiation from those of hemophilia, but here again the picture and diagnostic points are much the same as those for purpura. Severe hemorrhages associated with obstructive jaundice and due to the high bile salt content of the blood, need scarcely be confused with hemophilia. Bleeding of the newborn is often wrongly diagnosed as hemophilia, although such a mistake is easily avoided. This disease, while very similar to hemophilia in the type of bleeding seen, comes on only during the first week of life, while hemophilia very rarely manifests itself before the second year. There exists no known relationship between these two hemorrhagic diseases. Hemolytic streptococcic infections, particularly in the sockets of extracted teeth, may lead to such prolonged bleeding as to raise the question of hemophilia. None of the typical diagnostic points are to be found, however, and differentiation should be easy. Prolonged malnutrition in children at times brings on a bleeding tendency, but it is usually more of the purpuric type.

Prognosis.—The outlook for the hemophilic child in past decades was distinctly unfavorable. The mortality from hemorrhage was high during the years of childhood, while the nutritional state and physical development were usually retarded by the abnormal life of constant care and fear of hemorrhage. Later in life the repeated joint hemorrhages and low grade infection left these unfortunate persons with such deformities and limitation of motion that most of them remained semi-invalids for life. Only too frequently the betterment of bleeding tendency that came with adolescence and adult life was more than counterbalanced by the increase in joint distress from the more active life. In a great many cases, fortunately, the disease can be expected to decline in severity from the beginning of adolescence onward, so that its handicaps may eventually be outgrown, except for the joint involvements.

In more recent years the prognosis has assumed a much more hopeful character. Several new methods of treatment have robbed the hemorrhages of much of their danger, while better knowledge of nutrition and of its importance in this disease has made possible a more normal childhood development. In fact,

if the plan of treatment as outlined below is followed consistently, hemophilia should cease to be such a dreaded disease and such a scourge to its victims.

Treatment.—The treatment of hemophilia is to be divided into two parts: (1) the handling of the immediate hemorrhage and (2) prophylactic measures for lessening the future hazards of the disease. In the treatment of the immediate hemorrhage too much emphasis cannot be laid on one important point, *no time should be lost in applying the most effective means of hemostasis at hand.* Only too frequently it happens that little attention is paid to the slow blood loss from these patients for the first two or three days, the hope being that it will stop spontaneously. Due to the fact that prolonged blood loss often results in a hemorrhagic phase associated with the anemia and reduction in concentration of the blood clotting constituents, it is very important that prompt and energetic measures be instituted early in hemophilic hemorrhages. The first two days offer the greatest chance of prompt stoppage of the bleeding. Beyond this period great difficulty may be encountered and repeated blood transfusions required.

In handling a hemophilic hemorrhage the first step is, of course, the application of physical means of compression (packing, bandaging, surgical ligations, etc.) to slow the blood loss as much as possible, but at the same time instituting those forms of treatment best adapted to increase the coagulability of the blood. Many hemostatic and coagulant preparations have been marketed for this purpose, a few of which are sometimes effective. In general, those preparations depending only on cephalin for their effectiveness are not of much value in hemophilia, while those containing tissue fibrinogen, such as the lung, brain, or other tissue extracts, are more useful, either when given orally or subcutaneously. I personally favor the purified tissue fibrinogen rather than any of the crude tissue extracts. If administered orally, tissue fibrinogen should be given in 3 to 5 c.c. doses (of a 1.5 per cent solution) in cold water on an empty stomach, preferably one-half hour before meals and at midnight. This is necessary in order to approximate a continuous effect. Subcutaneously 1 to 2 c.c. doses should be given every eight hours for a continuous effect. In the beginning it is well to give the injections every two hours for four injections, before going to the eight-hour schedule. This gives a maximum effect in a minimum time. Administration of the coagulant should be continued for at least twenty-four hours after the bleeding has ceased. These same principles of administration would hold for any of the biologic coagulants in common use.

Other procedures also to be instituted at once include the administration of a high protein diet, as will be discussed later, the induction of a local skin protein reaction, provided the patient has been previously sensitized, and the subcutaneous injection of 0.1 to 0.3 c.c. of adrenalin solution every four hours except in cases where the bleeding is arterial. In case a local skin protein reaction can be elicited, this is usually sufficient to stop the bleeding, without the use of tissue fibrinogen or adrenalin. In every other case, however, all the other procedures previously mentioned should be used promptly and in conjunction, so as to avoid the greater difficulties that come with the prolonged blood loss.

In case the patient is seen for the first time only after he has been bleeding

for a number of days, then the procedure already mentioned should be begun at once, but with less hope of benefit. In this case one should prepare for blood transfusion as soon as possible. Repeated transfusions are often necessary at this stage, and should be used always in conjunction with the other procedures previously mentioned. One caution should be kept in mind, however. Transfusion should not be performed on a patient within eight hours after a previous dose of tissue fibrinogen, since this coagulant tends to intensify the transfusion reactions that sometimes occur. Tissue fibrinogen may be administered with safety, however, within an hour after the transfusion. It seems to make little difference whether the direct or the citrate method be used in performing the transfusion. This procedure, which is often a life-saving measure in the prolonged hemorrhages of hemophilia, is not justified during the first two days of bleeding. Other treatment is usually effective during this early stage and is more economical. It is always advisable to keep transfusion as a reserve measure as long as possible, since the patient so often considers his condition more critical when he learns that the physician is planning to resort to transfusion.

Treatment of hemophilic patients in a prophylactic way involves several separate procedures which are here detailed.

1. *Sensitization to a foreign protein* offers such an immediately effective means of treating hemorrhages when they occur, and of preventing their occurrence, that I give it first place in prophylaxis. Vines[23] discovered this method, and its effectiveness in increasing the coagulability of hemophilic blood has since been verified by the writer[15] and others.[6, 24, 25] It is effective in such a large percentage of hemophiliacs that no physician handling these patients is justified in failure to make proper use of it. The technic is as follows: The patient is sensitized to some foreign protein by intramuscular injection, I prefer sheep or hen serum, since they have practically no other therapeutic use. Three or four cubic centimeters of the serum are injected intramuscularly after making certain that the patient is not already sensitive to a drop administered intradermally. After the expiration of two weeks, intradermal injection of a drop of the serum is again made. The formation of the usual urticarial wheal denotes proper sensitivity, and studies of the blood coagulability before and after the induction of the local reaction will show a marked improvement. Not only is the clotting time much shortened, but one now finds the prothrombin to be much more nearly normal in its reactions, especially toward cephalin.[15] Should the clotting time not shorten sufficiently after the first skin reaction, one may repeat the intracutaneous injection for several successive weeks in different skin areas. Repeated local reactions do not tend to reduce the general sensitivity which usually lasts for a year or more and which may be subsequently renewed, using the same or a different protein. The production of a generalized systemic protein reaction is to be carefully avoided, since its effect is to produce a temporary hemorrhagic state which might prove disastrous to the hemophilic patient. Care should therefore be taken to make sure that the patient is not already sensitive to the serum to be injected intramuscularly. Likewise, it would be unfortunate if intended intradermal injections should be given subcutaneously instead. By

keeping all one's hemophilic patients sensitive to some foreign protein, at least during the time in life when their disease is most troublesome, one always has at hand an immediately effective method of treating hemorrhages as they occur. And if one desires to use the method for the prevention of bleeding, one needs only to induce the skin reactions as often as is indicated by observations of the blood coagulability.

2. The use of *high protein diet* is an important adjunct in prophylaxis as well as in treatment of the hemorrhages. It is definitely established that the absorption of protein food from the intestine is accompanied by an increased coagulability of the blood, so that the clotting time is usually shortened by 30 to 40 per cent for one to four hours after each meal containing protein.[26, 27] Use has been made of this fact in the treatment of hemorrhagic states with considerable success.[28, 29] It involves the administration of a moderate amount of protein in some form at each meal and the drinking of milk, cocoa, or egg nog between meals and at least once during the night. In this way the protein effect on blood coagulability is continuous and tends to be cumulative. A high carbohydrate, low protein diet has just the reverse effect on the blood and should be avoided in hemophilia. There are practically no contraindications to this diet in hemophilia, so that use should always be made of it for prophylaxis or treatment of hemorrhages. Should it give rise to too much intestinal putrefaction, relief can at once be obtained from the use of the following prescription:

	Kaolin	vi
	Aq. dest.	iv
	Syr. Tolu. q. s. ad	xii
M. Sig.:	One-half ounce each evening for adults.	
	One drachm each evening for children.	

3. *Theelin,* the follicular hormone now on the market, has been found by Birch[7] to be very effective in hemophilia. Wiedemer, in Cincinnati, has had occasion recently to verify the findings of Birch in this regard. The technic she has found most effective in adolescent patients consists in subcutaneous injections of 1 c.c. of theelin (Parke, Davis & Company) every second or third day. Within two or three weeks the clotting time usually has approached close to normal and can be kept there by two injections a week. Daily injections seem to be too frequent, as the clotting time tends to rise. In like manner weekly intervals are too long. There is no permanency to the effects, since the clotting time promptly begins to mount once they are discontinued. Coincident with the disappearance of the bleeding tendency under theelin, there usually occurs an improvement in nutrition with a gain in body weight. It is obvious that this treatment is not applicable for the arrest of an existing hemorrhage, on account of the long latent period. Its main usefulness will be found in the preparation of these patients for tooth extraction or minor surgical procedures and for use when a patient appears to be entering a hemorrhagic period. Its usefulness may, of course, be considerably increased as our knowledge of it grows with experience.

4. *Improvement in the nutritional state* of hemophilic patients is one step that should always receive careful consideration in outlining a plan of treatment.

Proper diet should be provided, keeping in mind the protein effect. Especial attention should be given to the vitamin content of the diet, particularly the vitamin B content (old nomenclature). A good adjunct for this purpose is the use of purified wheat hearts, such as may be obtained very cheaply from most milling companies. Such a product may easily be incorporated in the preparation of pancakes, bread, cooked breakfast cereals, etc. It is pleasant to use and provides an economical supply of important vitamins. There are much more expensive preparations on the market which one may use for this same purpose, if it is desired. Further stimulation of the metabolism by ultraviolet light or heliotherapy is helpful at times during the winter and spring months. One's effort should be directed mainly toward securing as nearly normal physical development in these children as is possible. As their nutritional state improves, so also does their bleeding tendency, in most instances.

5. *Removal of foci of infections* should receive careful attention for two reasons: first, because it will greatly aid in the desired nutritional improvement, and second, on account of its relation to the chronic arthritis so frequently present in these patients. Carious teeth and infected roots form the greatest danger in this regard. The fear of hemorrhage so often prevents these patients seeking or receiving proper dental care, with the result that advanced stages of infection and decay are usually present. It is altogether likely that it is these foci of infection, seeding into the hemorrhagic joints time after time, that prevent proper recovery following each joint hemorrhage and eventually lead to the bad deformities so often seen. With our present methods of preparing these patients for dental work, little difficulty should be encountered in eradicating foci of infection from the mouth. Infected sinuses should likewise receive proper attention, although surgical operations on the sinuses or tonsils should still be undertaken with a great deal of caution, no matter how well prepared the patient may appear. Restoration of joint function becomes an orthopedic problem only after all evident foci of infection have been removed and the patient's blood coagulability brought as near to normal as is possible by the previously described means.

One should bear in mind that the prophylactic measures outlined here do not conflict with one another, and may well be used in conjunction, especially if any operative procedures are contemplated. As previously stated, one need have little dread of hemophilia if the treatment measures given here are properly used. Since the best of them are of very recent application, more experience will be necessary before one can be certain just how completely these patients can be prepared for the surgical emergencies that at times arise.

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VARIATIONS IN THE DIAMETER OF THE GRANULOCYTES*

A Preliminary Study

J. W. Love, M.D., Cleveland, Ohio

SINCE the epochal discovery by Ehrlich[1] that blood smears could be stained with the analine dyes, and the modification of these stains by Jenner,[2] Pappenheim,[3] Romanowsky,[4] Giemsa,[5] and Wright,[6] increasing interest in the stained elements of the blood has been shown. From the pioneer work of these men there has come an increasing accuracy in the field of clinical hematology.

The object of the present study was to determine the diameter of the granular elements of the blood, and the normal distribution curve, to establish the mean average diameter of these cells as seen in stained smears, to note the variations from normal, and finally to see if any clinical application or correlation could be made of these conclusions.

Credit for the discovery of the white cell is generally given to the English physiologist, Hewson,[7] whose published work appeared in 1771. Little note was taken of these cells, however, until Schultze[8] in 1865 studied warm-stage preparations, differentiated the various types of white cells and gave us the first accurate measurements of them. It was about fifteen years later that Ehrlich added immeasureably to the vista of hematology by the use of analine dye stains for the study of blood films.

In all the work reported in this paper smears were made upon cover slips and stained with the familiar Wright's stain plus a buffer made of sodium and potassium phosphates as initiated by McJunkin.[9] The cover slips were mounted on slides with gum damar and the actual measurements were made by using a filar micrometer eyepiece. The calibration with the microscope drawtube at 160 mm., 1/12 mm. Zeiss lens oil immersion objective, was so standardized that 11 drum divisions measured one micron, that is, each drum division was equivalent to one-eleventh of a micron. Only those cells were measured which were entirely round, halo free, and had assumed the familiar "rounded-up" appearance in the film; those cells the outline of which was oval or otherwise distorted or deformed were particularly avoided as were the edges of the smear where malformations of the leucocyte are so often discovered.

Many authors give various figures for the size of the granulocyte (see Table I). Wright[6] in Nelson's Medicine gives 9 to 12 microns as the size of the polymorphonuclear granulocyte, no figure for the eosinophile, and 8 to 10 microns for the size of the basophilic cell. Jordan[10] says that the neutrophiles "range in size from 7.5 μ to 10 μ in diameter" and that the eosinophiles and basophiles are "slightly smaller." Maximow,[11] Bailey,[12] Halliburton,[13] and others give various other figures. None of these authors give any method for

*From the Department of Medicine, Cleveland Clinic.

arriving at these conclusions or quote any references concerning them. Valuable references are to be found, however in Bunting's[14] article in *Cowdry's Special Cytology,* Hoeber, 1928. Bunting states, "The range of variation in diameter (of neutrophiles) given by Schultze and commonly accepted is from 9 to 12 μ."

TABLE I

SIZE OF GRANULOCYTES AS REPORTED BY VARIOUS AUTHORS

AUTHOR	NEUTROPHILES	EOSINOPHILES	BASOPHILES
Bailey	8-12 μ	12-14 μ	8-10 μ
Barker	9-12 μ	sl larger	8-10 μ
Bunting	9-12 μ	10-12 μ	8-10 μ
Cummer	9-12 μ	sl larger	8-10 μ
Halliburton	9-12 μ	12-15 μ	10 μ
Jordan	7.5-10 μ	sl smaller	sl smaller
Maximow	10-12 μ	12 μ	10 μ
Ordway and Gorham	9-12 μ	.....	.....
Wright	9-12 μ	.....	10 μ

The present study has for its basis the measurement of the diameters of one hundred or more polymorphonuclear granulocytes in each of fifteen normal individuals, this number being chosen in order to insure measurement of a sufficient number to establish a normal. Of these fifteen normals eight were men and seven were women. These individuals were unselected except for general good health and no account was taken of age, size, or body weight. Naturally the figures obtained apply only in this climate.

It was found that there is no distinct difference in diameter of cells according to sex. The average diameter of the polymorphonuclear granulocytes for the series was 13.3 μ. None of the normals had a leucocytosis or a leucopenia. The diameter of many of the cells found was greater or less than the average diameter of 13.1 μ, but in series they remain remarkably close to this figure and it may be taken as a constant. The smallest polymorphonuclear cells were 8.8 μ and 9.1 μ in diameter but only one specimen of each of these was found; correspondingly, cells of 15 μ in diameter occurred several times but were distinctly rare in smears of normal blood.

The absolute spread of these cells, therefore, is from 8.8 μ to 15.8 μ, but these figures are outside the usual limits found and the great majority show little variation. In typical distribution curves the cell diameters range from 11.2 μ to 15.0 μ and these latter figures are used in the construction of distribution curves to represent the normal variations in diameter (see Chart 1).

The diameter of the normal eosinophile in stained cover slip preparations taken from a somewhat larger group of normals in order to count one hundred such cells, was found to be 14.3 μ. This is the average diameter of 100 normal eosinophiles and no smear of an eosinophilia was included. The size of the normal basophile is based upon the measurement of 25 normal specimens of this cell and was found to be 13.2 μ for adults of the series. The range in diameters for the eosinophile was from 12.2 μ to 16.7 μ; for the basophile 10.9 μ to 14.6 μ.

It was thought advisable to give a figure for the diameter of the normal non-filamentous polymorphonuclear cell, since this has been the subject of recent studies and to see that it did not vary consistently from the size of other polymorphonuclear cells. The average diameter of 100 such cells selected at random from normals was found to be 13.0 μ.

The normal diameters for leucocytes of this series therefore are as follows: polymorphonuclear granulocytes 13.1 μ; eosinophiles 14.3 μ, and basophiles 13.2 μ; these are accepted as constants in order to compare with the diameters of the cells measured in the abnormal conditions to follow.

The first of these conditions in which the diameter of the granulocyte was measured was *leucocytosis*. Five cases were chosen at random. They represent some of the ordinary conditions in which we have come to expect an elevation of the leucocytes to occur and represent leucocytosis in varying degrees ranging from a moderate elevation of 9,700 to that of a rather severe degree, 45,000 white

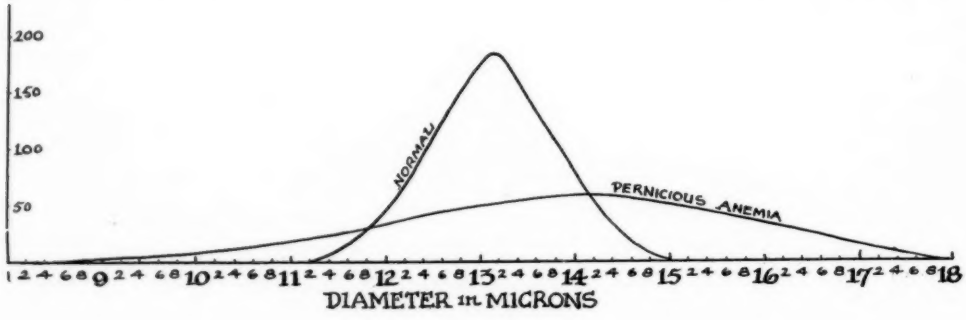

Chart 1.

cells. In these conditions the polymorphonuclear cell was present in predominance, 82 per cent to 96 per cent (Table II).

TABLE II

DIAMETER OF POLYMORPHONUCLEAR GRANULOCYTES IN LEUCOCYTOSIS

CASE	DIAGNOSIS	LEUCOCYTOSIS	DIAMETER
1	Infectious arthritis	17,000	13.5 μ
2	Sinusitis	20,000	13.1 μ
3	Pyelitis	25,000	13.1 μ
4	Bronchopneumonia	33,000	13.0 μ
5	Lobar pneumonia	45,000	12.8 μ

The average diameter of the polymorphonuclear cell in these conditions is 13.0 μ which is so close to the normal figure as to be without significance. One would believe, therefore, that stimulation of the bone marrow in the customary degree causes no appreciable change in the diameter of the granulocytes no matter how rapidly they are added to the blood stream.

The second abnormal condition to be investigated was *leucopenia*. In one case in which the total white count was 2,300 cells resulting from multiple abscess formation, the average white cell diameter was nearly normal, 12.7 μ. Three cases of malaria in which the total counts were 1,600, 1,700, and 2,300

white blood cells respectively had average cell diameters considerably below the normal figure, the first being 11.6 μ, the second 11.5 μ, and the third 11.3 μ. A case in which the total white cell count was 500 was seen in a condition which resembled malignant neutropenia, but too few cells were observed to make proper measurements and no other suitable case has been seen. Although further work must be done on the group of leucopenias, nevertheless, one might justifiably reach the conclusion that depression of the bone marrow results in the production of a granulocyte of somewhat smaller diameter than normal, especially as seen in malaria. The leucopenia accompanying pernicious anemia will be considered later.

The third abnormal condition to be studied was *polycythemia vera*. Recently some interesting work has been done by Cross[15] on the white cells in this condition. The diameter of the white cells was measured in three cases at this Clinic. All these cases showed an increase in red blood corpuscles and hemoglobin, and an increased blood volume was found by actual measurement. The average diameter of the polymorphonuclear white cells was found to be 13.1 μ, nor did it vary from this although the number of white cells ranged between 8,500 and 20,900. No case in which a marked leucopenia was present was at hand for study.

Fourth, the white cell diameter in the condition which is commonly designated simple *achlorhydric anemia* was investigated. In studies in five typical cases no significant change from the normal was noted. This result might have been predicted inasmuch as the pathologic physiology in this condition seems to concern hemoglobin anabolism rather than bone marrow deficiency.

Fifth, in addition to the three cases of polycythemia vera showing variation in the diameter of the red blood corpuscles, one case of hepatic disease was seen which showed a marked *megalocytosis* and the diameter of the white blood cells was accordingly measured. Here again, however, no change from the average diameter of 13.1 μ was found.

Hence, it may be seen that the diameter of the white blood cells is a very stable measurement and in practically all the above pathologic conditions, with the exception of malarial leucopenia, no change in diameter could be found. It might be added at this point that the number of lobules of the nucleus seems to bear no relation to the diameter of the cells in any of the measurements encountered.

Sixth, cases of *pernicious anemia* were studied both before and after treatment with a view to establishing whether the variations in size which the red blood cells are known to undergo might be paralleled in the white blood cells. Routine measurements were made in the case of patients as they presented themselves at the Clinic. They were all suffering from various grades of anemia and all showed some depression of their hemopoietic activities, hence all the measurements in the beginning were actually made on leucopenias. The white blood cell count ranged from a total of 1,600 cells up to 2,500 cells. In ten typical cases it was found that the diameter of the polymorphonuclear granulocyte was markedly increased, 14.0 μ. This increase is striking in some cases as individual cells of 17.5 μ to 18.0 μ in diameter were not uncommonly observed.

There are, however, cells of greatly diminished diameter, as of 8.5 μ to 9.0 μ. Thus the spread of white blood cells in pernicious anemia is quite wide (broad base to the distribution curve) ranging from 8.5 μ to 18.0 μ. These variations are shown graphically in Chart 1.

Five cases of pernicious anemia were studied both before and after the red blood cell count and hemoglobin had risen from quite a low figure to approxi-

TABLE III

AVERAGE DIAMETER OF WHITE BLOOD CELLS IN PERNICIOUS ANEMIA

CASE	BEFORE TREATMENT	AFTER TREATMENT
1	14.3 μ	13.1 μ
2	13.8 μ	13.1 μ
3	14.2 μ	13.4 μ
4	14.0 μ	12.8 μ
5	14.0 μ	13.3 μ
Averages	14.0 μ	13.1 μ

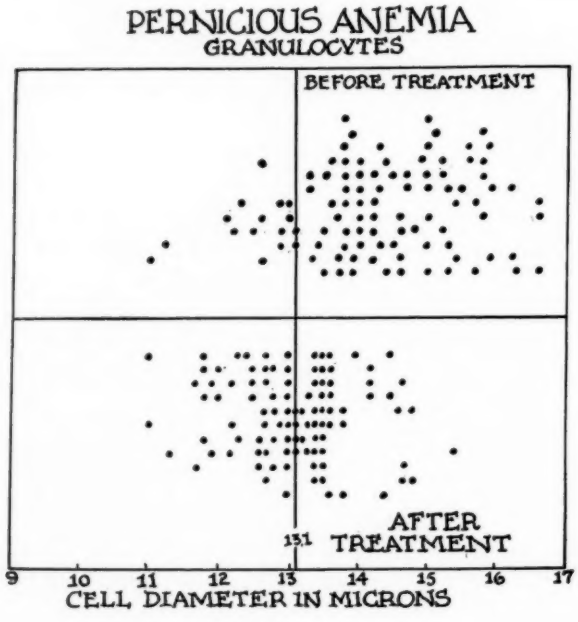

Chart 2.

mately normal (Table III). The white cells were found to be large at first, averaging 14.0 μ in diameter but returned to their normal figure when improvement had occurred. This condition of the white cells has been discovered only in true Addisonian anemia and not in any other type of disease so far encountered. Although this increase in the diameter of white cells, megalopolycytosis, does not seem great, it is present in all the cases studied and will perhaps constitute another factor in enabling us to understand this malady and perhaps throw some light upon what occurs in a remission. This change from abnormal (increased) diameter to normal is shown graphically in one case in Chart 2.

SUMMARY AND CONCLUSIONS

Measurement of the diameter of white blood cells in stained blood films in the case of fifteen normal adults shows the mean average diameter of the cells to be 13.1 μ for polymorphonuclears, 14.3 μ for eosinophiles, and 13.2 μ for basophiles. These figures are well above the commonly quoted normals which seem to be based upon the work done by Schultze in 1865. For comparative studies on changing white cell diameters these figures may be considered as constants.

The variation in diameter (spread) of the normal polymorphonuclear granulocyte is within the limits of from 11.6 μ to 14.6 μ usually. Contrary to this, pernicious anemia is accompanied by a granulocyte of larger diameter than normal and the spread in distribution curves of untreated cases is greatly increased, ranging from 8.5 μ to 18.0 μ for polymorphonuclear cells. During a remission these cells return to their normal diameter.

Significant variations from the normal white cell diameter occur only in certain abnormal blood states and no change is found in the ordinary leucocytosis or leucopenia.

Variations of rather marked degree do occur, however, when there is marked depression of the bone marrow as in the leucopenia accompanying malaria and pernicious anemia.

No explanation for these phenomena is offered at this time.

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(*Symposium to be continued in July issue*)

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EDITORIAL

The March of Hematology

JOHN HUNTER[1] in his Treatise on the Blood, remarked that the early observers of the blood cells "probably imagined more than they saw." Hunter's book testifies as to the scant knowledge of the blood in his time, since his discussions centered largely around the coagulation, the buffy coat, the cause of the difference in color of venous and arterial blood and similar topics. The development of hematology as we now know it began in the middle of the nineteenth century when Vierordt,[2] in 1851, first enumerated the erythrocytes and thus stimulated work in blood counting. The original crude and laborious methods were rapidly improved so that the counting of cells soon became a clinical procedure. Malassez'[3] monograph on the enumeration of the red cells appeared in 1873. The counting chamber devised by Gowers[4] in 1877, remained the standard until recent years.

Hemoglobin was discovered and isolated in crystalline form by Funke[5] in 1851, and Welcher[6] in 1854 made the first clinical estimation of hemoglobin by

comparing a fixed dilution of the unknown blood with dilutions of normal blood. Interest in hemoglobin was widespread and quite complete analyses of hemoglobin crystals soon were made. Accurate knowledge of hemoglobin is due largely to the early work of Hoppe-Seyler[7] who showed that hemoglobin combines with oxygen in the lungs to form oxyhemoglobin which in turn gives up the oxygen in the tissues again to form hemoglobin. He thus identified the fundamental importance of the blood pigments in tissue metabolism. Preyer's[8] monograph on the blood pigments appeared in 1871. The first monograph on clinical hemoglobinometry by Leichtenstern[9] was published in 1878 and a number of hemoglobinometers were suggested before 1880.

With the development of accurate methods for the counting of the blood cells and the determination of hemoglobin, clinical applications were made quickly. In 1864, Welcher,[10] a student of Vierordt's, wrote a very comprehensive article on the "size, the number, the volume, the surface, and the color of the blood corpuscles of man and animals." The first clinical hematology by Hayem[11] appeared in 1878. Soon afterwards, (1883) Laache's[12] classic monograph on anemia was published. The names of Vierordt, Welcher, Hoppe-Seyler, Malassez, Hayem, and Gowers are closely identified with the discovery of the fundamentals of hematology.

The next important development was the introduction of Ehrlich's[13] method for staining blood film with analin dyes.[13] This opened an entirely new field for investigation of the blood and made possible the morphologic study of the different types of cells. The final chapter in the development of fundamental methods for blood study was Hedin's[14] suggestion concerning the use of centrifugal force to separate cells and plasma in the hematocrit.

Since then, many new refinements in blood study have been and still are being added. We now constantly use reticulocyte counts, determine the bile pigments, utilize knowledge concerning the embryology of the blood, measure cells, differentiate cells by supravital, oxidase and many other stains, and employ numerous procedures almost routinely in the study of blood. Any known blood dyscrasia can be most carefully investigated from the laboratory standpoint, if one but uses the measures now available.

In no field of medicine today is more intensive investigation being made or is more rapid progress resulting than in the acquisition of new knowledge concerning normal and pathologic conditions of the blood. The medical journals contain many articles on every phase of the subject and many papers are contributed to the programs of the various societies. This added interest is due in large measure to improvements in methods of treatment of the anemias and of other blood diseases for which credit is due largely to Whipple and to Minot and Murphy. Much remains to be done. The leucemias and some other blood dyscrasias continue a challenge in regard to treatment. Every worker in this important field, however, can well be proud of the march of hematology.

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R. L. H.

CORRESPONDENCE

Dear Sir:

In my paper on Bacteriophage in Clinical Medicine which appeared in the April number of the Journal of Laboratory and Clinical Medicine, I made the following statement:

"The early experiments of d'Herelle with fowl typhoid were not sufficiently extensive to be conclusive. His later work with barbone in buffaloes was apparently satisfactory although Cowles and Hale make the statement that in a personal communication d'Herelle claims the opposite."

This statement is misleading since the work to which Cowles and Hale referred concerned therapeutic application, the results of which had not been published owing to the small number of animals concerned. The work which d'Herelle reported concerned immunization of buffaloes and it has been amply confirmed by LeLouet. Hence, there has been no change in the published papers of d'Herelle on this subject as was implied in my statement.

I would greatly appreciate it if you could find space for this correction in a forthcoming number of the Journal, also for the statement of my regret that there should have been a misunderstanding on my part of the statement of Cowles and Hale.

(Signed) N. W. LARKUM